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

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Barroga C, Hu Y, Deshmukh V, Hood J. Discovery of an Intra-Articular Injection Small Molecule Inhibitor of the Wnt Pathway (SM04690) As a Potential Disease Modifying Treatment for Knee Osteoarthritis. Presented at: 2015 American College of Rheumatology (ACR)/ Association of Rheumatology Health Professionals (ARHP) Annual Meeting; 2015 Nov 6-11; San Francisco, CA.

Disclosures

- Charlene Barroga, Ph.D.
  - Financial disclosure: Samumed, LLC; salary and equity
- Yong Hu, Ph.D.
  - Financial disclosure: Samumed, LLC; salary and equity
- Vishal Deshmukh, Ph.D.
  - Financial disclosure: Samumed, LLC; salary and equity
- John D. Hood, Ph.D.
  - Financial disclosure: Samumed, LLC; salary and equity

All authors are employees of Samumed, LLC

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Evidence-Based Medicine

Wnt Regulates Chondrogenesis

- JA Rudnicki & AM Brown. Inhibition of Chondrogenesis by Wnt Gene Expression in Vivo and in Vitro. *Dev Biol.* 1997. 185:104-18.

Wnt Polymorphisms Associated with Osteoarthritis

- J Loughlin. Polymorphism in signal transduction is a major route through which osteoarthritis susceptibility is acting. *Curr Opin Rheumatol.* 2005. 17:629-33.

Wnt Involved in Osteoarthritis

- van den Bosch MH, Blom AB, Sloetjes AW, Koenders MI, van de Loo FA, van den Berg WB, van Lent PL, van der Kraan PM. Induction of Canonical Wnt Signaling by Synovial Overexpression of Selected Wnts Leads to Protease Activity and Early Osteoarthritis-Like Cartilage Damage. *Am J Pathol.* 2015. 185:1970-80.

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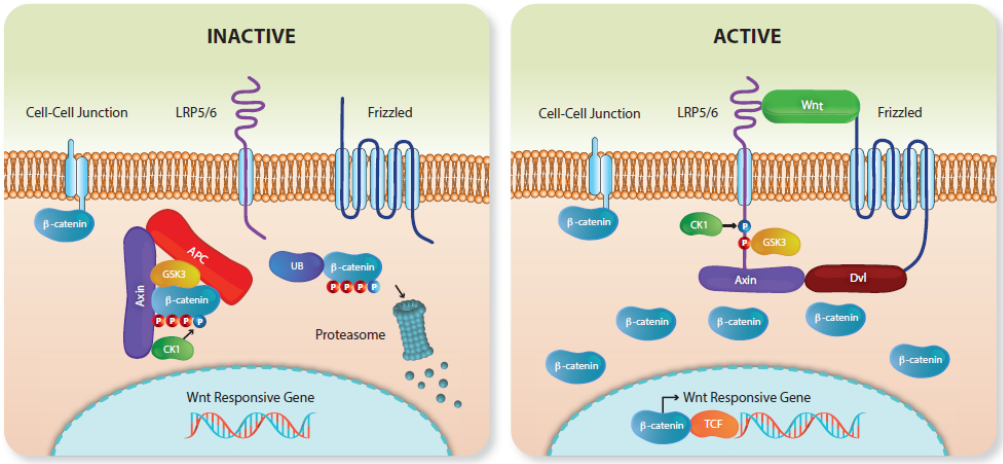
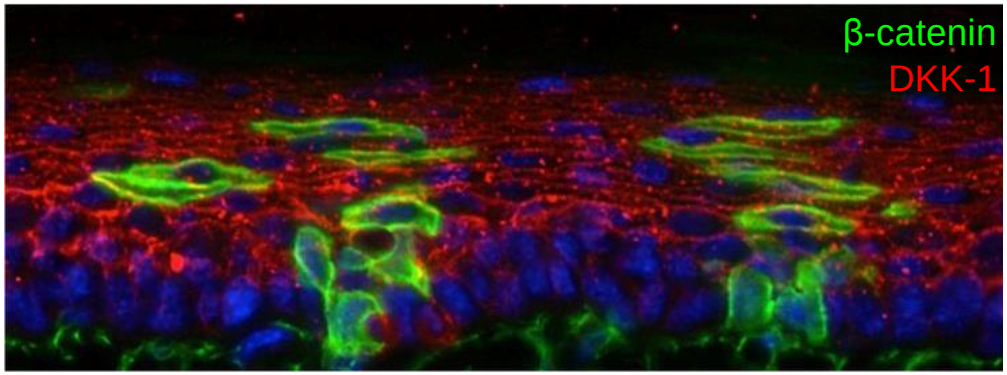
Wnt Signaling Pathway

- The Wnt (wingless & int1) pathway is highly conserved across all animals
- Controls stem cell differentiation
- Implicated in tissue development & regeneration





Wnt pathway plays a key role in tissue repair and regeneration

Reference: Image from Lim, et al. *Science.* 2013;342:1226-30.

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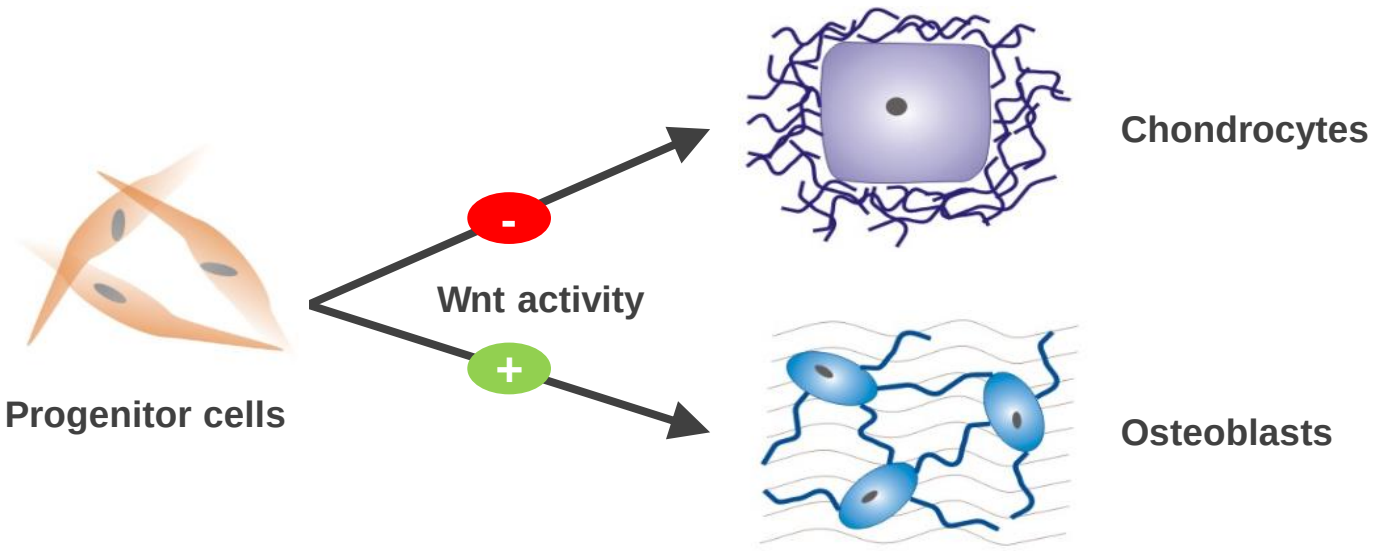
Wnt Pathway and Osteoarthritis



- Increased Wnt signaling contributes to the pathophysiology of OA.[1-5]

Hypothesis: Inhibiting the Wnt pathway regenerates cartilage and treats osteoarthritis

Figure adapted from www.york.ac.uk

References:
1. Blom AB, et al. *Arthritis Rheum*. 2009;60(2):501-12.
2. Im GI, et al. *Biotechnol Lett*. 2011;33(5):1061-8.
3. Loughlin J. *Curr Opin Rheumatol*. 2005;17(5):629-33.
4. Rudnicki JA & Brown AM. *Dev Biol*. 1997;185(1):104-18.
5. Thomas RS, et al. *Arthritis Res Ther*. 2011;13(6):R203.

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Proposed Therapy: SM04690

- SM04690 drug product has the following properties:
  - Small molecule
  - Inhibitor of the Wnt signaling pathway
  - Intra-articular injection

SM04690 has the potential to regenerate cartilage and treat OA

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SM04690 *In Vitro* Studies

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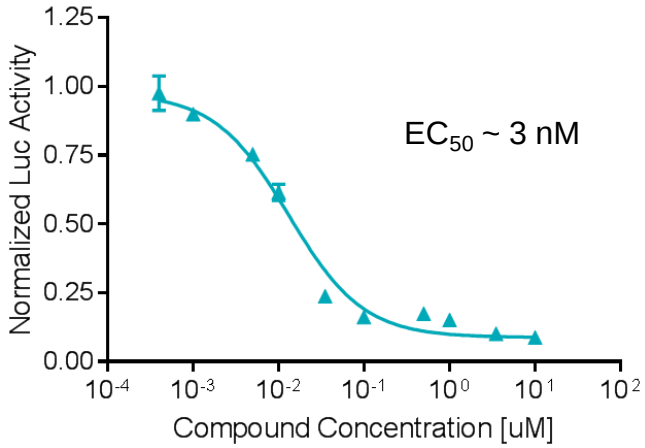
Wnt Response and Wnt Gene Expression

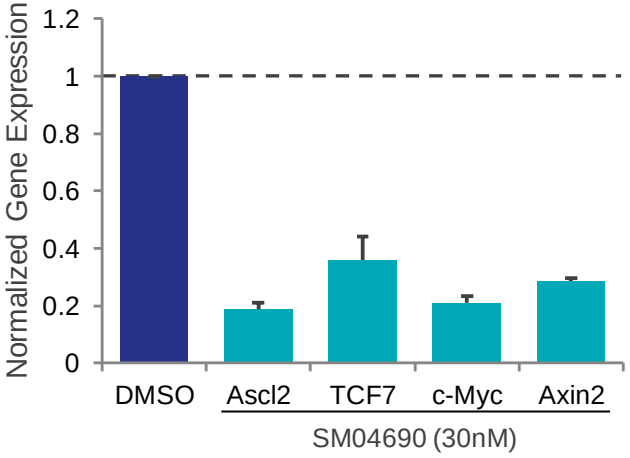
Cellular assay – Colon Cancer Cells:

- High turnover cell line, tightly regulated by Wnt pathway
- Stable expression of Wnt reporter
- Luciferase based readout for Wnt activity



Cellular assay – Human Mesenchymal Stem Cells:

- hMSCs treated with Wnt proteins and SM04690
- Expression of Wnt pathway genes measured by qPCR
- DMSO treated cells used as control
- Significant downregulation of Wnt genes at 48hrs



SM04690 is a potent inhibitor of the Wnt pathway

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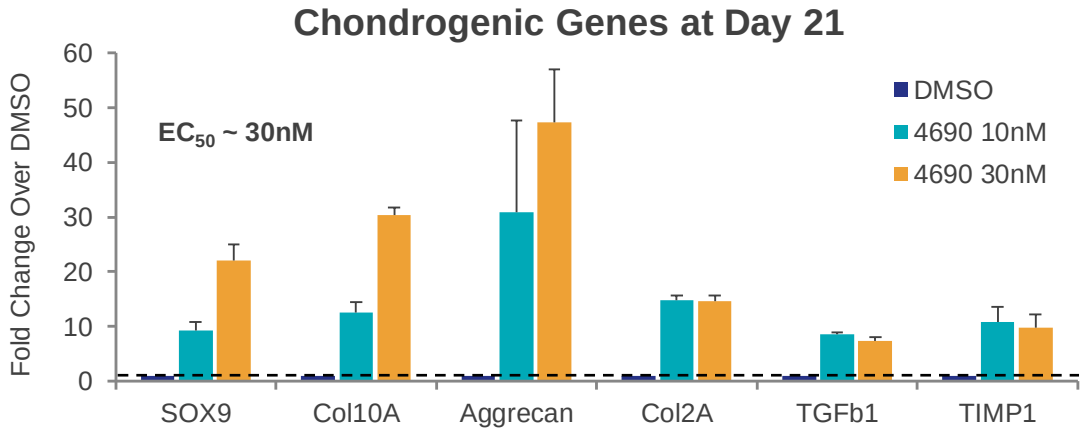
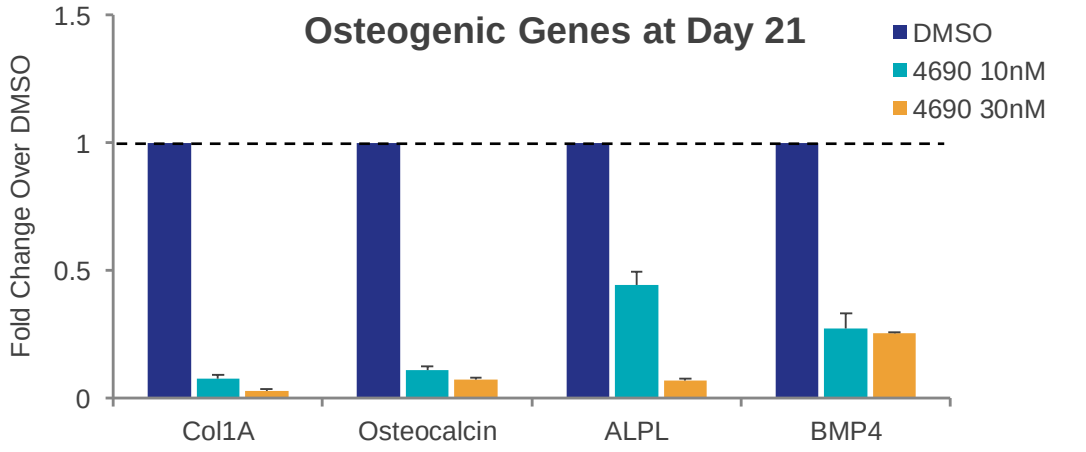
Chondrogenic and Osteogenic Gene Expression

Cellular assay – hMSCs:

- Treated with SM04690
- qPCR performed at 21 days





SM04690 upregulated chondrogenic gene expression and downregulates osteogenic gene expression

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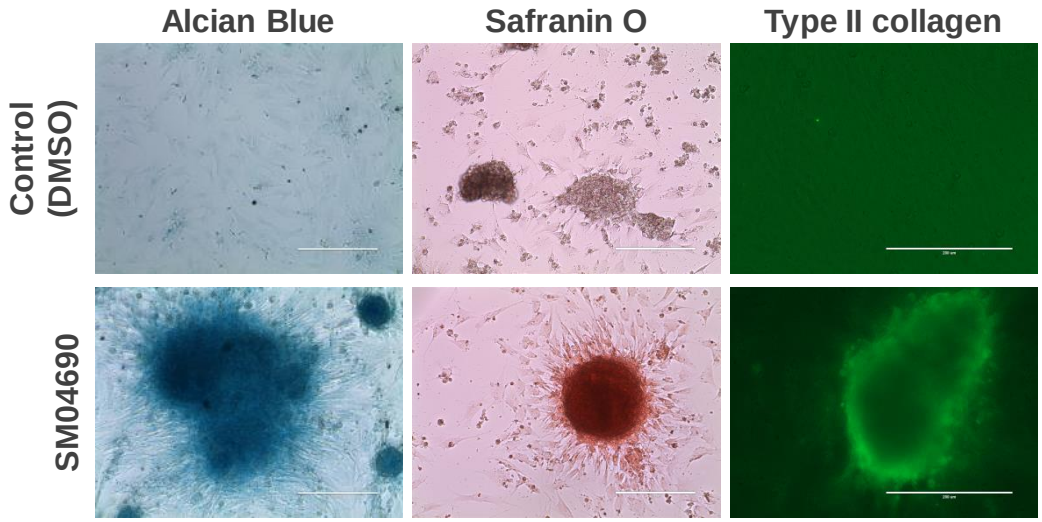
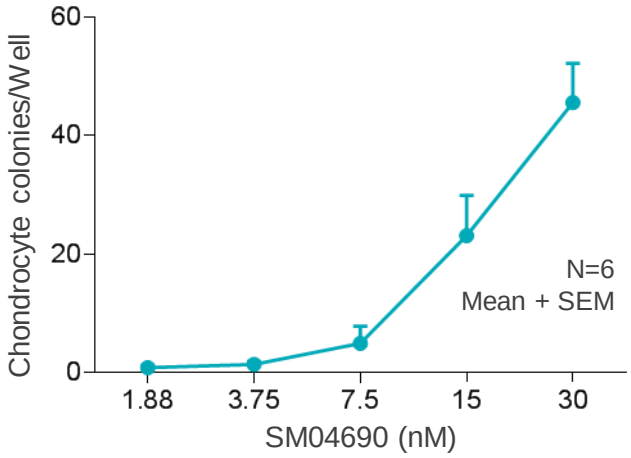
Chondrogenesis

Cellular assay – hMSCs:

- Treated with SM04690
- Cells fixed and stained with Alcian Blue, Safranin O, and various chondrocyte markers
- Chondrogenesis quantified as number of stained chondrocyte colonies per well
- Dose dependent chondrogenesis demonstrated





SM04690 induces chondrogenesis

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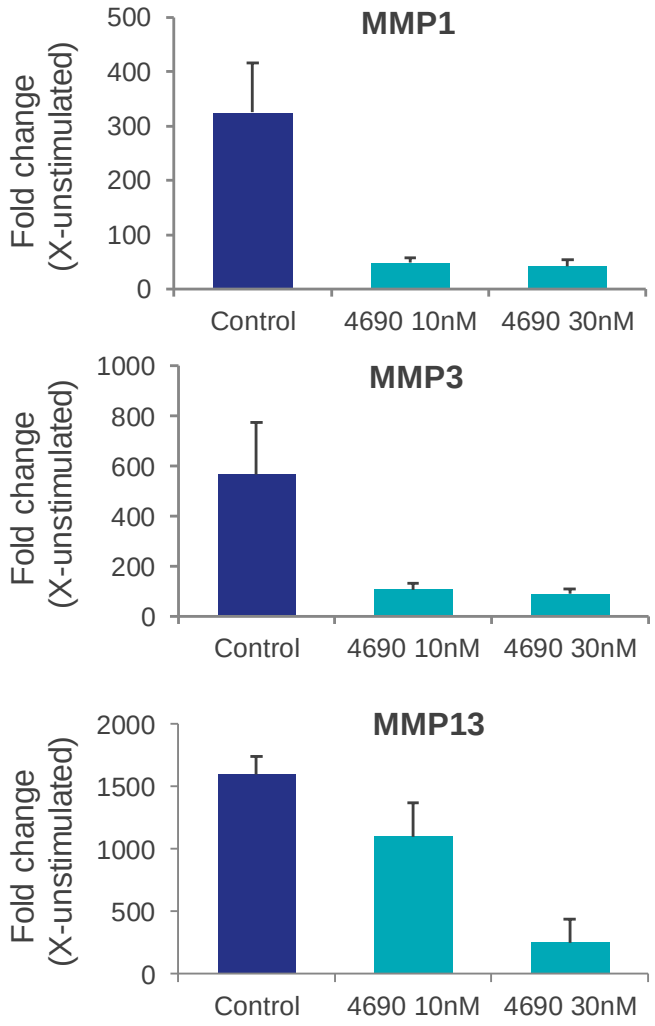
Effect on Protease Production

- In OA, cytokines induce cartilage catabolic enzymes (Matrix Metalloproteinases, mainly MMP1, MMP3, and MMP13)
- In OA, MMPs cause degenerative tissue remodeling

Cellular assay – human chondrocytes:

- Treated with TNFα + Oncostatin M to induce protease release
- Then treated with SM04690
- qPCR performed for MMP1, MMP3 and MMP13
- Dose dependent inhibition of protease expression demonstrated



SM04690 inhibits protease production

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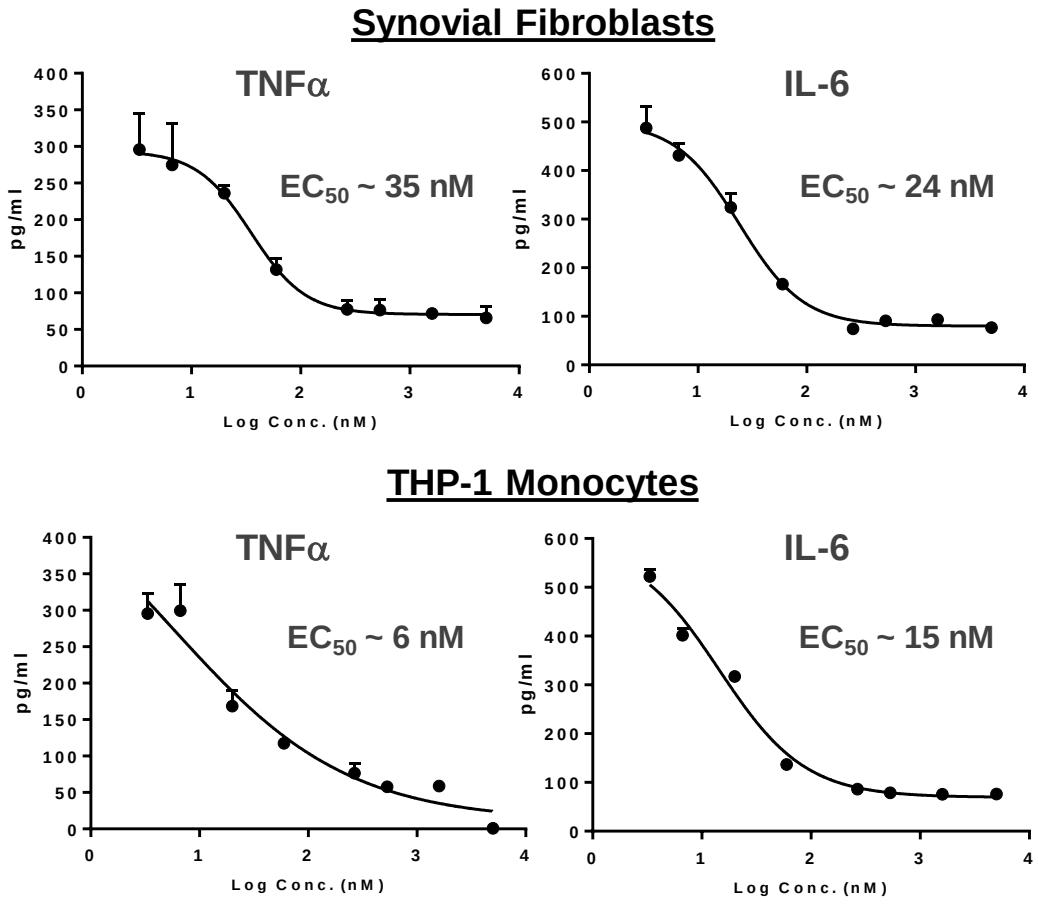
Production of Inflammatory Cytokines

- TNFα and IL-6 are the most common inflammatory cytokines
- TNFα and IL-6 play a major role in the pathogenesis of OA

Cellular assay:

- Synovial fibroblasts stimulated with IL1β and THP-1 monocytes stimulated with LPS to induce cytokine production
- Then treated with SM04690
- Cytokine production quantified by ELISA
- Dose dependent inhibition of both TNFα and IL-6 production demonstrated in both cell types



SM04690 suppresses inflammatory cytokine production

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SM04690 *In Vivo* Studies

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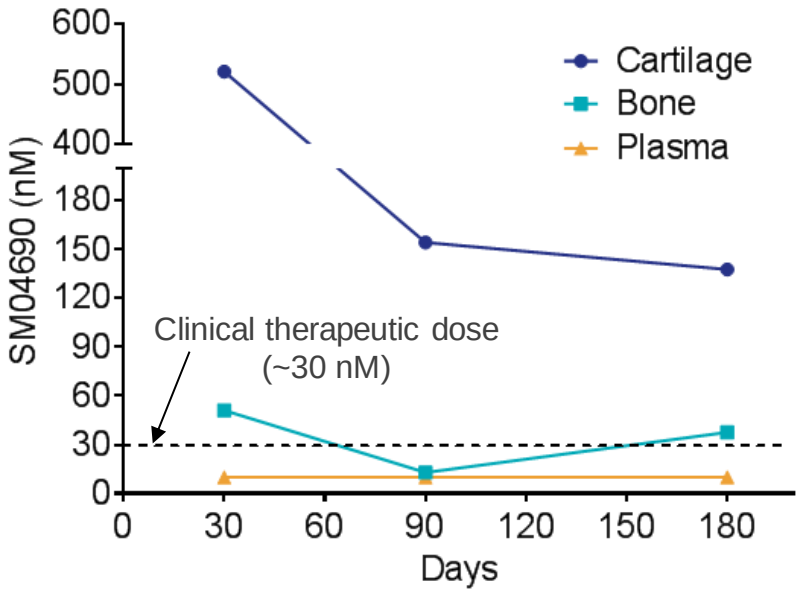
Pharmacokinetics

Rats (Sprague Dawley):

- Single intra-articular injection
- 3 rats (2 knees/rat) at each 30, 90, 180 day time points.
- Compound is retained in joint above the target concentration level (~30 nM)
- Compound is undetectable in plasma at all time points



SM04690 had sustained local exposure and no systemic exposure

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Toxicology

Rats (Sprague Dawley) and Dogs (Beagle):

Intra-articular (IA) injection

- Single and multiple intra-articular injections
- Right knee joint histologically evaluated for inflammation, cartilage health, bone density, etc.
- **No systemic toxicity** - body weight, target or non-target organs effects, ECG and clinical pathology at doses up to 400X the lowest therapeutic dose
- Local inflammation (at the injection site) at doses >1,400X the therapeutic dose
- No detectable systemic exposure at any dose at all time points

SM04690 shows no observable systemic toxicity after IA injection

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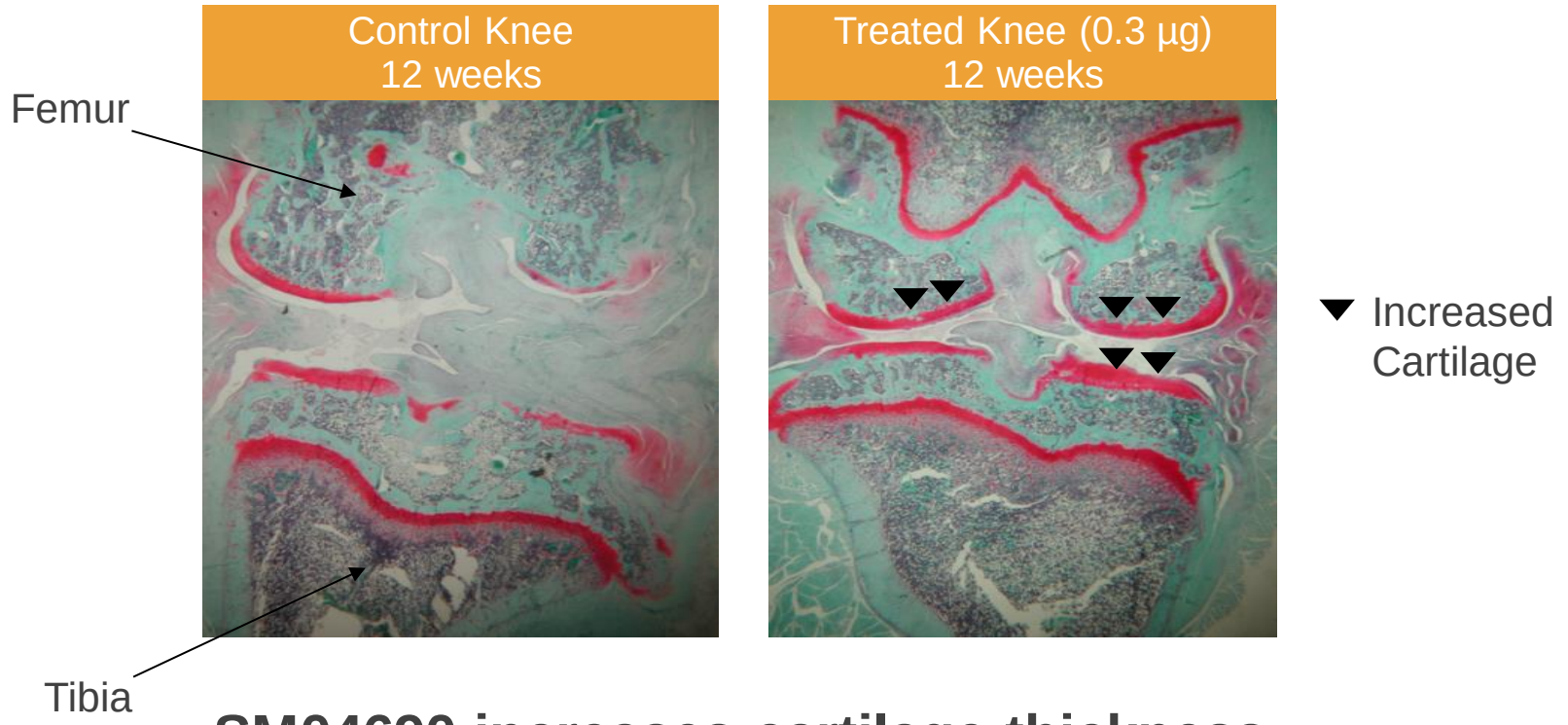
Efficacy

- Rat instability model - anterior cruciate ligament transection combined with medial meniscectomy
- Allow cartilage degeneration for 2 weeks
    - Inject SM04690 (0.3 mg) intra-articularly
    - Evaluate joints by histology after 12 weeks
- Safranin O-stained sections from the rat knee analyzed 3 months post-surgery for OA cartilage pathology using the OARSI scoring system
- Increased cartilage thickness, decreased fissures, and subchondral bone remodeling observed with a single intra-articular injection of SM04690



SM04690 increases cartilage thickness

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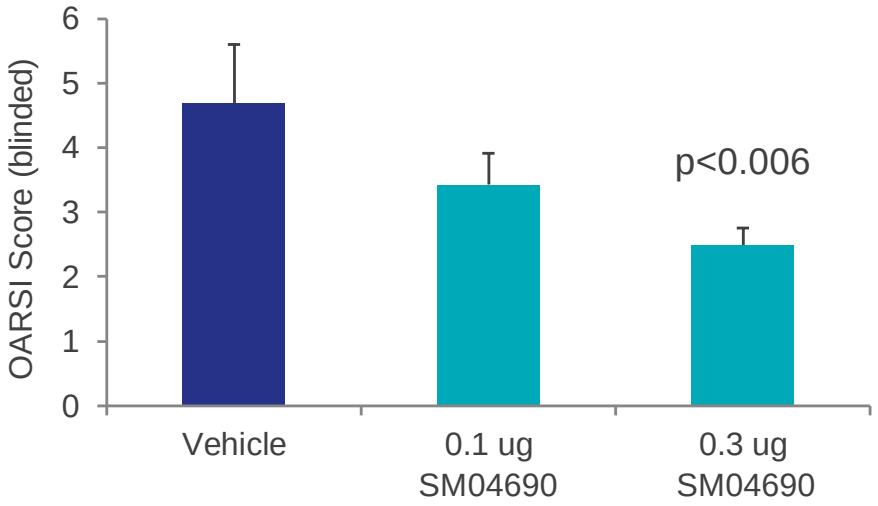
OARSI Score

- Safranin O-stained sections from the rat knee scored (blinded) for OA cartilage pathology using the OARSI system
- OARSI score measures cartilage matrix loss, fissures, subchondral bone remodeling and bone cyst formation
- Dose dependent reduction in total OARSI score (against vehicle) demonstrated, indicating improved overall cartilage health



SM04690 significantly improves joint health

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Summary

- SM04690 is a potent inhibitor of the Wnt pathway
  - Induces chondrogenesis
  - Inhibits protease production and inflammatory cytokine production
  - Has sustained local availability and no systemic exposure
  - No observable systemic toxicity
  - Regenerates cartilage

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Next Steps

- Osteoarthritis of the knee
  - Completed Phase 1 study (N=60)
  - Phase 2 study (N=400) is on-going

- Plans to expand into OA of other joints
  - Hip
  - Shoulder

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Thank you

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