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

Charlene Barroga, Ph.D., Yong Hu, Ph.D., Vishal Deshmukh, Ph.D., and John Hood, Ph.D.

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
Evidence-Based Medicine

Wnt Regulates Chondrogenesis


Wnt Polymorphisms Associated with Osteoarthritis


Wnt Involved in Osteoarthritis

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


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

Hypothesis: Inhibiting the Wnt pathway regenerates cartilage and treats osteoarthritis

References:
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
SM04690 \textit{In Vitro} Studies
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

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

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**

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
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

SM04690 In Vivo Studies
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

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

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

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

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
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
Thank you