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

Nancy E. Lane, Vishal Deshmukh, Charlene Barroga, Haide Hu, KC Sunil and Yusuf Yazici
Disclosures

• Nancy E. Lane, MD
  • Financial disclosure: Consultant for Samumed and Regeneron

• Vishal Deshmukh, Ph.D.
  • Financial disclosure: Samumed, LLC; salary and equity

• Charlene Barroga, Ph.D.
  • Financial disclosure: Samumed, LLC; salary and equity

• Yong Hu, Ph.D.
  • Financial disclosure: Former employee of Samumed, LLC; equity

• K.C. Sunil
  • Financial disclosure: Employee of Samumed, LLS equity

• Yusuf Yazici, M.D.
  • Financial disclosure: Samumed, LLC; salary and equity
Wnt is a Critical Pathway in Osteoarthritis

- OA is characterized by increased subchondral bone and reduced cartilage.
- Wnt proteins are over-expressed and more active in OA joints.
- Increased Wnt signaling leads to cartilage catabolism and increased bone formation:
  - Progenitor cells form osteoblasts instead of chondrocytes.
  - Increased proteases (MMP) are released leading to cartilage catabolism.
- Inhibiting Wnt signaling may break the cycle and shift from cartilage degeneration to regeneration.

MMP – Matrix Metalloprotease
4. van den Bosch et al. 2015. Am J Pathol.
Proposed Therapy: SM04690

• SM04690 is a small molecule Wnt signaling inhibitor in development for the treatment of OA
In Vitro Studies: SM04690 suppressed inflammatory cytokines

Cellular assays:
- Synovial fibroblasts stimulated with LPS and peripheral blood mononuclear cells (PBMCs) stimulated with super antigen (sAg).
- SM04690 significantly inhibited pro-inflammatory cytokine secretion.

SM04690 showed potent anti-inflammatory activity in vitro

n=3 replicates, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001.
In Vitro Studies: SM04690 inhibited protease production in Chondrocytes

- In OA, cytokines induce cartilage matrix metalloproteases (MMPs) through Wnt signaling
- SM04690 demonstrated a dose dependent inhibition of protease expression

**Cellular assay – human chondrocytes:**

In Vitro Studies: SM04690 inhibited GAG & Nitric Oxide secretion

- Glycosaminoglycan (GAG) are components of cartilage matrix
- Secreted/extracellular GAG = cartilage breakdown
- Inhibition of GAG and Nitric Oxide (NO) secretion demonstrated

Cellular assay – human chondrocytes:

Induce catabolism

| IL-1β or TNFα + Oncostatin M |

Treat

| SM04690 or Control |

Measure

| Secreted GAG and NO |

SM04690 protected chondrocytes from catabolic breakdown

GAG

TNF-α + OM
IL-1β
SM04690 (30nM)

*N* p<0.05
**p<0.01
***p<0.001

Nitric Oxide

TNF-α + OM
IL-1β
SM04690 (30nM)
In Vitro Studies: SM04690 Induced Human MSCs to differentiate into functional chondrocytes

21 day cellular assay – hMSCs:
- Treated with SM04690 every 7 days
- qPCR analysis showed increased chondrogenic and decreased osteogenic gene expression as compared to DMSO control.
- Increased sulfated glycosaminoglycans (sGAG) with SM04690 treatment

**SOX9**

**Aggrecan**

**Col2A**

**TGFB1**

**TIMP1**

21 day cellular assay – hMSCs:
- Treated with SM04690 every 7 days
- qPCR analysis showed increased chondrogenic and decreased osteogenic gene expression as compared to DMSO control.
- Increased sulfated glycosaminoglycans (sGAG) with SM04690 treatment

**Chondrogenic Genes**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Fold Change Over DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOX9</td>
<td><strong>20</strong></td>
</tr>
<tr>
<td>Aggrecan</td>
<td><strong>40</strong></td>
</tr>
<tr>
<td>Col2A</td>
<td><strong>60</strong></td>
</tr>
<tr>
<td>TGFB1</td>
<td><strong>80</strong></td>
</tr>
<tr>
<td>TIMP1</td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

**sGAG**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Fold Change Over DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>20</td>
</tr>
<tr>
<td>SM04690 (30nM)</td>
<td><strong>40</strong></td>
</tr>
</tbody>
</table>

**Osteogenic Genes**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Fold Change Over DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Col1A</td>
<td><strong>20</strong></td>
</tr>
<tr>
<td>Osteocalcin</td>
<td><strong>40</strong></td>
</tr>
<tr>
<td>ALPL</td>
<td><strong>60</strong></td>
</tr>
<tr>
<td>BMP4</td>
<td><strong>80</strong></td>
</tr>
</tbody>
</table>

* p<0.05
** p<0.01
*** p<0.001
Hypothesis:

Inhibition of the Wnt Signalling Pathway will both prevent cartilage deterioration and regenerate cartilage in preclinical models of OA.
Efficacy: Rat Instability Model of OA

**Rat ACLT+pMMx model:**

- Anterior cruciate ligament transection (ACLT) combined with partial medial meniscectomy
- Cartilage degeneration is present within 1-2 weeks
- Treatment: SM04690 administered intra-articularly at 1wk post-injury at (0.1 μg, 0.3 μg, 1 μg)
- Joint histology performed 4 (day 35) and 12 (day 90) weeks after treatment.
Chondrocyte Gene expression showed Wnt pathway proteins in OA model

- SM04690 treatment decreased the expression of Wnt signaling activators and downstream genes, e.g. Wnt3a, Dvl, Axin2, TCF4, TCF7, APC, GSK3β, CyclinD1 etc. as compared to vehicle.
- SM04690 treatment increased the expression of Wnt pathway inhibitors, e.g. DKK1, 2, WIF1 etc. as compared to vehicle.

Week 5

![Graph showing relative expression of Axin2 and β-catenin](image)

**Axin 2**
- Relative Expression
- Vehicle vs SM04690

**β-catenin**
- Relative Expression
- Vehicle vs SM04690

SM04690 inhibited Wnt signaling *in vivo* in cartilage

* p<0.05    N=7 rats for vehicle, N=8 rats for SM04690
Cartilage Gene Expression found SMO4690 Decreased Cartilage Degradation Enzymes in the ACLT + pMMx model of OA

- qPCR evaluation of protease enzymes in cartilage
- Decreased protease expression in cartilage with SM04690 treatment

**Week 5**

![Bar chart showing relative expression of MMP1, MMP3, MMP13, and ADAMTS5 in Vehicle and SM04690 (0.3ug) groups.]

* p<0.05  ** p<0.01  *** p<0.001  N=12 rats/group

**SM04690 protected cartilage from catabolic breakdown**
SM04690 Appeared to Stimulate cartilage formation in the ACLT + pMMx model of OA

- qPCR evaluation of cartilage composition markers
- Increased expression of cartilage composition markers with SM04690 treatment
  - Increased sulfated glycosaminoglycans (sGAG) - cartilage matrix
- No change in Col10a (hypertrophic marker)

**Week 5**

![Graph showing relative expression of Col2a1, COMP, Aggrecan, and Col10a](image)

![Graph showing sGAG levels](image)

* p<0.05  *** p<0.001  ns - not significant  N=12 rats/group
Biomarkers and OARSI scores

- Decreased serum COMP and increased serum PIIANP observed with SM04690 treatment
- Safranin O-stained sections from the rat knee scored (blinded) using OARSI system
- OARSI cartilage pathology score measures cartilage matrix loss, fissures and subchondral bone remodeling, based on stage and grade of cartilage damage

---

**COMP**

<table>
<thead>
<tr>
<th>Week 3</th>
<th>Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>2.5 ± 0.3</td>
</tr>
</tbody>
</table>

**PIIANP**

<table>
<thead>
<tr>
<th>Week 4</th>
<th>Week 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>1000 ± 50</td>
</tr>
</tbody>
</table>

**OARSI score**

<table>
<thead>
<tr>
<th>Week 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
</tr>
<tr>
<td>SM04690 (0.1µg)</td>
</tr>
<tr>
<td>SM04690 (0.3µg)</td>
</tr>
</tbody>
</table>

*p<0.05  **p<0.01  N=12 rats/group*
Histology of the Cartilage

- Safranin O-stained sections from the rat knee analyzed 13 weeks post-surgery for OA cartilage pathology
- Increased cartilage thickness and decreased cartilage fissures observed with a single intra-articular injection of SM04690

Control

![Control Image]

SMO4690

![SMO4690 Image]
SM04690-01 Efficacy Outcomes
WOMAC total [0-96] – change from baseline (mITT)

Exploratory analysis
SM04690-01 Efficacy Outcomes

OMERACT-OARSI strict response (mITT)

OMERACT-OARSI strict response: ≥ 50% improvement and corresponding ≥ 20 point (scaled 0-100) improvement in either WOMAC pain or function subscale
Summary

• Wnt signaling is a critical pathway in osteoarthritis

• In preclinical models, SM04690:
  – Inhibited inflammatory cytokine and protease production
  – Induced chondrogenesis

• Clinical data
  – Phase 1: A single intra-articular OA knee injection with SM04690 appeared safe, well-tolerated and improved pain and function compared to the placebo.
  – The Clinical Development Program is ongoing with Phase 2 studies that are now in progress to determine the efficacy of SMO4690 as an OA disease modifying agent.
Thank you