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

Vishal Deshmukh, Ph.D., Charlene Barroga, Ph.D., Yong Hu, Ph.D., John Hood, Ph.D., and Yusuf Yazici, M.D.
Disclosures

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

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

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

• John D. Hood, Ph.D.
  o Financial disclosure: Co-founder and former employee of Samumed, LLC

• Yusuf Yazici, M.D.
  o Financial disclosure: Samumed, LLC; salary and equity
Pathophysiology of Osteoarthritis

- Mechanical forces and inflammation result in degenerative tissue remodeling in OA.
- Induce cartilage catabolic enzymes - matrix metalloproteinases (MMPs), aggrecanases, etc.
- Cartilage loss and subchondral bone remodeling

Wnt Pathway and Osteoarthritis

• The Wnt signaling pathway is involved in stem cell control and regeneration of tissues
• Increased Wnt signaling contributes to the pathophysiology of OA
• Wnt signaling is involved in increased bone formation and cartilage breakdown
• Progenitor cells reside in the synovium and subchondral bone

Hypothesis: Inhibiting the Wnt Pathway protects and regenerates cartilage


Proposed Therapy: SM04690

• SM04690 is a small molecule Wnt inhibitor in development for the treatment of OA

• SM04690 demonstrated the following properties in pre-clinical studies:
  – Decreased inflammation
  – Decreased cartilage degradation
  – Regenerated cartilage
  – Sustained local exposure and no observable systemic toxicity
In vitro Efficacy- SM04690
Decreased inflammation:
SM04690 suppressed inflammatory cytokines

- IL1β, TNFα and IL-6 are associated with the pathophysiology of OA\(^1\)

**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 TNF\(\alpha\) and IL-6 production demonstrated in both cell types

Decreased cartilage degradation: SM04690 inhibited protease production

- In OA, cytokines induce cartilage catabolic enzymes
- Increased Wnt signaling increases protease expression
- Dose dependent inhibition of protease expression demonstrated

**Cellular assay – human chondrocytes:**

<table>
<thead>
<tr>
<th>Induce proteases</th>
<th>Treat</th>
<th>Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFα + Oncostatin M</td>
<td>SM04690 or Control</td>
<td>qPCR: MMP 1, 3, &amp; 13</td>
</tr>
</tbody>
</table>

Decreased cartilage degradation: SM04690 inhibited GAG and Nitric Oxide release

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

**Cellular assay – human chondrocytes:**

**Induce catabolism**
- IL1β or TNFα + Oncostatin M

**Treat**
- SM04690 or Control

**Measure**
- Secreted GAG and NO

SM04690 protected chondrocytes from catabolic breakdown
Regenerated cartilage: SM04690 induced functional chondrogenesis

21 day cellular assay – hMSCs:

- Treated with SM04690 every 7 days
- Cells stained for biomarkers and gene expression measured by qPCR
- Increased sulfated glycosaminoglycans (sGAG) with SM04690 treatment
- Functional chondrocytes-cartilage matrix synthesis

Chondrogenic Genes

<table>
<thead>
<tr>
<th></th>
<th>DMSO</th>
<th>SM04690 (30nM)</th>
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<tbody>
<tr>
<td>SOX9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aggrecan</td>
<td>**</td>
<td>***</td>
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<tr>
<td>Col2A</td>
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<td>**</td>
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<tr>
<td>TGFβ1</td>
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<td>***</td>
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<td>TIMP1</td>
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GAG

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<tr>
<th></th>
<th>DMSO</th>
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<tbody>
<tr>
<td>GAG (μg/mg)</td>
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Osteogenic Genes

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<tr>
<th></th>
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<tbody>
<tr>
<td>Col1A</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>Osteocalcin</td>
<td></td>
<td>***</td>
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<tr>
<td>ALPL</td>
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<td>***</td>
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<tr>
<td>BMP4</td>
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* *p<0.05
** p<0.01
*** p<0.001
In vivo Efficacy
SM04690 had sustained local exposure and no systemic toxicity

Rats (Sprague Dawley):
- Single intra-articular injection (0.3ug)
- Compound is retained in joint above the target concentration level (~30 nM) for >6 months
- Compound is undetectable in plasma at all time points

Intra-articular (IA) injection in Rats (Sprague Dawley) and Dogs (Beagle):
- Single or multiple (6 or 9 once-monthly) IA injections
- **No systemic toxicity** - body weight, target or non-target organ effects, ECG and clinical pathology at doses up to 400X the expected clinical dose
Decreased cartilage degradation: ACLT + pMMx model of OA

- Rat ACLT + pMMx model- anterior cruciate ligament transection (ACLT) combined with partial medial meniscectomy (pMMx)
- Inject SM04690 single dose, intra-articular after 1 week
- Rat knee analyzed 5 and 13 weeks post-surgery for OA cartilage pathology

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

Week 5

SM04690 protected cartilage from catabolic breakdown
SM04690 regenerated cartilage: ACLT + pMMx model of OA

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

**Week 5**

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>SM04690 (0.3ug)</th>
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<tbody>
<tr>
<td><strong>Relative Expression</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Col2a1</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>COMP</td>
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<td>Aggrecan</td>
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<td>Col10a</td>
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<tbody>
<tr>
<td><strong>GAG (ug/g)</strong></td>
<td></td>
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</table>

* p<0.05  *** p<0.001  ns- not significant  N=12 rats/group

**SM04690 induced chondrocyte and cartilage matrix production**
SM04690 regenerated cartilage: Improved OA 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

SM04690 improved joint health

* p<0.05    ** p<0.01
N=12 rats/group
SM04690 regenerated cartilage

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

SM04690 increased cartilage thickness
Summary

- Wnt signaling is a critical pathway in osteoarthritis

- In preclinical models, SM04690:
  - Inhibited inflammatory cytokine and protease production
  - Induced chondrogenesis
  - Had sustained local availability and no systemic exposure
  - Had no observable systemic toxicity
Thank you

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