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

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Disclosures

- 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
- Yusuf Yazici, M.D.
  - Financial disclosure: Samumed, LLC; salary and equity
Samumed overview

- Samumed, LLC started operations in 2008 with headquarters in San Diego, CA.
- The Wnt pathway is one of the primary signaling pathways regulating the regeneration and repair of cells. Samumed has developed compounds that modulate the Wnt pathway and may lead to recovery and restoration of diseased tissues.

### Pipeline

<table>
<thead>
<tr>
<th>Pre-Clinical</th>
<th>Phase I</th>
<th>Phase II</th>
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</thead>
<tbody>
<tr>
<td>Osteoarthritis (OA of the Knee)</td>
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<tr>
<td>Androgenetic Alopecia (AGA)</td>
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<tr>
<td>Idiopathic Pulmonary Fibrosis (IPF)</td>
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<td>Psoriasis (PsO)</td>
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<tr>
<td>Tendinopathy</td>
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<tr>
<td>Scleroderma (SCL)</td>
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<tr>
<td>Degenerative Disc Disease (DDD)</td>
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<tr>
<td>Colorectal, Pancreatic, Liver and Gastric Cancers</td>
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<td>Osteoarthritis (OA) other indications</td>
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<td>Alzheimer’s Disease (AD)</td>
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</table>
The Wnt (wingless & int1) pathway is highly conserved across all animals.

- Involved in development of multiple tissues.
- Plays a critical role in self renewal and fate determination of stem cells.

Wnt pathway plays a key role in tissue repair and regeneration.

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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

**Figure adapted from Bush J & Beier F. Nature Med. 2013;19(6):667-9.**
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 signaling inhibitor in development for the treatment of OA

- SM04690 demonstrated the following properties in pre-clinical studies:
  - Inhibited Wnt signaling *in vitro* and *in vivo*
  - Decreased inflammation
  - Decreased cartilage degradation
  - Regenerated cartilage
  - Sustained local exposure and no observable systemic toxicity
*In vitro* Efficacy - SM04690
SM04690 Inhibited Wnt Signaling

- SM04690 was a potent inhibitor (EC50= 19nM) of the TCF/LEF reporter in SW480 colon cancer cells (fig. A)
- Downstream targets of Wnt signaling measured by qRT-PCR (fig. B) and Western Blot (fig. C) in human mesenchymal stem cells (hMSC)
- SM04690 inhibited expression of Wnt targets in hMSC

A.

B.

C.

\[ \text{Normalized Luciferase Activity} \]

\[ \text{Log Conc. (nM)} \]

\[ \text{Normalized Luciferase Activity} \]

\[ \text{Relative Expression} \]

\[ \text{DMSO} \]

\[ \text{SM04690 (30nM)} \]

\[ \beta\text{-catenin} \]

\[ \text{c-Myc} \]

\[ \text{Axin2} \]

\[ \text{Lef1} \]

\[ \text{TCF7L2} \]

\[ \text{TCF7} \]

\[ \text{Ascl1} \]

\[ \beta\text{-Actin} \]

n=3 replicates, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001
Decreased inflammation: SM04690 suppressed inflammatory cytokines

- IL-1β, IL-6, IL-8 and TNF-α are associated with the pathophysiology of OA

**Cellular assay:**
- Synovial fibroblasts stimulated with IL-1β and THP-1 monocytes stimulated with LPS to induce cytokine production, then treated with SM04690
- Cytokine production quantified by ELISA and qRT-PCR
- Dose dependent inhibition of IL-1β, IL-6, IL-8, and TNF-α production demonstrated in both cell types

IL-6 **EC**\textsubscript{50} = 24nM; TNF-α **EC**\textsubscript{50} = 35nM

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

Decreased inflammation: SM04690 suppressed inflammatory cytokines

**Cellular assays:**

- Synovial fibroblasts stimulated with LPS and peripheral blood mononuclear cells (PBMCs) stimulated with super antigen (sAg).
- SM04690 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.
Decreased inflammation: 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), with 0=weak activity and 5=highly potent activity.
- SM04690 demonstrated comparable or better activity than the two standard-of-care drugs across several anti-inflammatory assays.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Immunosuppression</th>
<th>Anti-Inflammatory</th>
<th>Th1/Th2/Th17 Inhibition</th>
<th>Cell Cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T Cell</td>
<td>B cell</td>
<td>Th1/Th17</td>
<td>Th1</td>
</tr>
<tr>
<td>SM04690 (37 nM)</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Cyclosporin A (120nM)</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>2</td>
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<tr>
<td>Prednisolone (120nM)</td>
<td>0</td>
<td>0</td>
<td>1</td>
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PBMC: peripheral blood mononuclear cells; HDF: human dermal fibroblasts; EC: endothelial cells; LPS: lipopolysaccharide
Decreased cartilage degradation: SM04690 inhibited protease production

- In OA, cytokines induce cartilage matrix metalloproteases (MMPs)
- Increased Wnt signaling increases protease expression\(^1\)
- SM04690 demonstrated dose dependent inhibition of protease expression

**Cellular assay – human chondrocytes:**

- Induce proteases: TNFα + Oncostatin M
- Treat: SM04690 or Control
- Measure: qPCR: MMP 1, 3, & 13

Decreased cartilage degradation: 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
Regenerated cartilage: SM04690 induced functional chondrogenesis

21 day cellular assay- hMSCs:
• hMSCs treated with SM04690 every 7 days
• Cells stained for biomarkers of chondrocyte differentiation
• SM04690 induced differentiation of hMSCs into chondrocytes

n=9 replicates, Mean ± SEM, *p<0.05, ***p<0.001.
Regenerated cartilage: SM04690 induced functional chondrogenesis

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
- Functional cartilage matrix synthesis demonstrated

Chondrogenic Genes

<table>
<thead>
<tr>
<th>Gene</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>
</tr>
<tr>
<td>Col2A</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>TGFb1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIMP1</td>
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sGAG

<table>
<thead>
<tr>
<th>DMSO</th>
<th>SM04690 (30nM)</th>
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<tbody>
<tr>
<td>GAG (µg/mg)</td>
<td>*</td>
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Osteogenic Genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>DMSO</th>
<th>SM04690 (30nM)</th>
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<tbody>
<tr>
<td>Col1A</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Osteocalcin</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>ALPL</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>BMP4</td>
<td>**</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
Efficacy: Rat Instability Model of OA

**Rat ACLT+pMMx model:**
- Anterior cruciate ligament transection (ACLT) combined with partial medial meniscectomy
- Cartilage degeneration within 1-2 weeks
- Injected SM04690 intra-articularly at 1wk (0.1 μg, 0.3 μg, 1 μg)
- Joint histology performed 4 and 12 weeks after injection
SM04690 inhibited Wnt signaling in cartilage in the ACLT + pMMx model of OA

- 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.

**SM04690 inhibited Wnt signaling *in vivo* in cartilage**

* p<0.05    N=7 rats for vehicle, N=8 rats for SM04690
Decreased cartilage degradation: ACLT + pMMx model of OA

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

Week 5

<table>
<thead>
<tr>
<th>Protein</th>
<th>Vehicle</th>
<th>SM04690 (0.3ug)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP1</td>
<td>1.0</td>
<td>0.7</td>
</tr>
<tr>
<td>MMP3</td>
<td>1.0</td>
<td>0.7</td>
</tr>
<tr>
<td>MMP13</td>
<td>1.0</td>
<td>0.7</td>
</tr>
<tr>
<td>ADAMTS5</td>
<td>1.0</td>
<td>0.7</td>
</tr>
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</table>

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

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

![Graphs showing relative expression and sGAG levels with vehicle and SM04690 (0.3ug) treatments.]

* 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

• Clinical data
  – Phase 1: A single intra-articular OA knee injection with SM04690 appeared safe, well-tolerated and potentially effective as a disease-modifying OA drug
  – Phase 2: ongoing
Thank you