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

Vishal Deshmukh PhD, Charlene Barroga PhD, Carine Bossard PhD, Sunil KC PhD, Tim Seo MS, Melinda Pedraza BS, Maureen Ibanez MS, Kevin Chiu BS, Long Do PhD, Shawn Cho MS, Luis Dellamary BS, Josh Stewart BS, Haide Hu PhD, Betty Tam PhD, John Hood PhD, Yusuf Yazici MD
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. (2013) Nature Med.
Wnt signaling is a critical pathway in OA: Cartilage catabolism

- OA is characterized by increased subchondral bone and reduced cartilage\(^1\)
- Wnt proteins are over-expressed and more active in OA joints\(^2\)
- Wnt pathway mutations (e.g. FrzB) are associated with OA\(^3\)
- Mechanical stress and inflammation increase Wnt pathway activity in the joint\(^3,4\)
- Increased Wnt signaling leads to cartilage catabolism\(^5\)
  - Proteases (MMPs, ADAMTs) are released leading to cartilage catabolism

MMP – Matrix Metalloprotease
ADAMT – A Disintegrin and Metalloproteinase with Thrombospondin motifs

Hypothesis: Modulation of the Wnt signaling pathway protects, and may regenerate, cartilage.
Proposed therapy: SM04690, a small molecule Wnt pathway inhibitor

- SM04690 is a small molecule Wnt signaling pathway inhibitor in development for the treatment of OA
- SM04690 demonstrated the following properties in pre-clinical studies:
  - Inhibited Wnt signaling \textit{in vitro} and \textit{in vivo}
  - Regenerated cartilage
  - Decreased cartilage catabolism
  - Decreased inflammation
  - Demonstrated sustained local exposure and no observable systemic toxicity
In vitro efficacy – SM04690
**Wnt signaling inhibition:**
SM04690 was a potent inhibitor in SW480 cells and hMSCs

- SM04690 inhibited TCF/LEF-reporter in SW480 cells (EC\textsubscript{50}=19nM). No effect on SV40-reporter
- SM04690 inhibited Wnt pathway gene expression in human mesenchymal stem cells (hMSCs)
- SM04690 was 50-500 fold more potent than previously reported Wnt pathway inhibitors

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC\textsubscript{50} (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SM04690</td>
<td>19.53</td>
</tr>
<tr>
<td>FH-535</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>IWR-1</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>CX-4945</td>
<td>2812</td>
</tr>
<tr>
<td>KY02111</td>
<td>&gt;10000</td>
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<tr>
<td>ICG-001</td>
<td>1428</td>
</tr>
<tr>
<td>iCRT14</td>
<td>&gt;10000</td>
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</tbody>
</table>

n=6, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001
Wnt signaling inhibition: SM04690 inhibited Wnt3a- & CHIR- induced Wnt signaling

- SM04690 was a potent inhibitor of Wnt pathway gene expression (Axin2, TCF7, Lef1) in hMSCs stimulated with Wnt3a or GSK3β inhibitor (CHIR-99021)
- SM04690 inhibited the expression of several Wnt pathway genes measured by RNA-seq

![Graphs showing relative expression of AXIN2, TCF7, and LEF1 genes under different conditions.](image)

n=4, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001
n=6 (pooled as n=3)
Decreased cartilage catabolism: SM04690 inhibited protease production

- In OA, cytokines induce cartilage catabolic enzymes
- Upregulated Wnt signaling increases protease expression\(^1\)
- SM04690 demonstrated dose-dependent inhibition of protease expression

Cellular assay – human chondrocytes:

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

n=3, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001

Regenerated cartilage: SM04690 induced chondrocyte differentiation

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

n=9, 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
- SM04690 treatment increased sulfated glycosaminoglycans (sGAG)
- Demonstrated functional cartilage matrix synthesis

Chondrogenic Genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>DMSO</th>
<th>SM04690 (30nM)</th>
</tr>
</thead>
<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>
<td></td>
<td></td>
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</table>

sGAG

<table>
<thead>
<tr>
<th>Condition</th>
<th>DMSO</th>
<th>SM04690 (30nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAG</td>
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</tbody>
</table>

Osteogenic Genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>DMSO</th>
<th>SM04690 (30nM)</th>
</tr>
</thead>
<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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</tr>
</tbody>
</table>

n=3, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001
Anti-inflammatory properties: SM04690 decreased pro-inflammatory cytokines

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

**Cellular assay:**
- Synovial fibroblasts stimulated with IL-1β or LPS to induce cytokine production, then treated with SM04690
- Dose dependent inhibition of IL-6 and TNF-α production, as well as several pro-inflammatory cytokines
- Mechanism under investigation

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**Log Conc. (nM)**

**Cytokine levels (pg/ml)**

IL-6 EC\(_{50}\) = 24nM; TNF-α EC\(_{50}\) = 35nM

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**Synovial Fibroblasts stimulated with LPS**

- IL-1β, IL-2, IL-5, IL-6, IL-8, TNF-α, IFN-γ
- DMSO, SM04690 (30 nM)
- Normalized fold change

\(n=3, \text{ Mean } \pm \text{ SEM, } \ast p<0.05, \ast\ast p<0.01, \ast\ast\ast p<0.001\)

In vivo studies – SM04690
Pharmacokinetics and toxicology: 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 tissue 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
Effects on OA in vivo:
SM04690 efficacy in a rat OA model

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
Wnt signaling in ACLT + pMMx model: SM04690 inhibited Wnt signaling in cartilage

SM04690 (compared to vehicle):
- decreased endogenous Wnt signaling activators and downstream gene expression
- increased expression of Wnt pathway inhibitory genes
- inhibited β-catenin nuclear localization in articular chondrocytes

Week 13

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>SM04690 (0.3 μg)</th>
</tr>
</thead>
</table>

**Total cellular β-catenin**

![Intensity Graph](image)

**Nuclear β-catenin**

![Intensity Graph](image)
Protected cartilage from catabolism: SM04690 decreased cartilage degrading proteases

- qPCR evaluation of protease enzymes in cartilage

SM04690 (compared to vehicle):
- decreased protease expression in cartilage, likely protecting cartilage from catabolism

Week 5
Protease Genes

<table>
<thead>
<tr>
<th>Protease Gene</th>
<th>Vehicle</th>
<th>SM04690 (0.3ug)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP1</td>
<td><img src="MMP1.png" alt="Graph" /></td>
<td><img src="MMP1.png" alt="Graph" /></td>
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<tr>
<td>MMP3</td>
<td><img src="MMP3.png" alt="Graph" /></td>
<td><img src="MMP3.png" alt="Graph" /></td>
</tr>
<tr>
<td>MMP13</td>
<td><img src="MMP13.png" alt="Graph" /></td>
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<tr>
<td>ADAMTS5</td>
<td><img src="ADAMTS5.png" alt="Graph" /></td>
<td><img src="ADAMTS5.png" alt="Graph" /></td>
</tr>
</tbody>
</table>

n=12, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001
Regenerated cartilage: SM04690 induced chondrogenesis and cartilage matrix production

- qPCR evaluation of cartilage production markers in cartilage 5 weeks after ACLT+pMMx

SM04690 (compared to vehicle):
- increased expression of cartilage markers
- increased sulfated glycosaminoglycans (sGAG) - cartilage matrix
- no change in Col10a (hypertrophic marker)

Week 5

**Chondrogenic Genes**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Vehicle</th>
<th>SM04690 (0.3ug)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Col2a1</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>COMP</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Aggrecan</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>Col10a</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

**GAG**

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>SM04690 (0.3ug)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sGAG (ug/g)</td>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>

n=12, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001, ns- not significant
Regenerated cartilage: SM04690 increased cartilage density and thickness

- Histology samples 13 weeks after ACLT+pMMx
- Safranin O-stained sections from the rat knee analyzed for OA cartilage pathology
- Increased Safranin O staining, cartilage thickness and decreased fissures observed with a single intra-articular injection of SM04690

![Images of histology samples comparing vehicle-treated and SM04690-treated knees at 13 weeks]

**Vehicle Treated Knee**
- 13 weeks

**SM04690 (0.3µg) Treated Knee**
- 13 weeks

Graphs showing:
- Cartilage thickness (µm) with **p<0.01**
- Safranin O intensity/area with ***p<0.001**

n=12, Mean ± SEM, **p<0.01, ***p<0.001
Regenerated cartilage:
SM04690 improved OARSI scores and OA biomarkers

- 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 (compared to vehicle):
- Decreased OARSI score.
- Decreased serum COMP (catabolic marker) and increased serum PIIANP (anabolic marker).

### Graphs

**OARSI Score**
- Week 13

**COMP**
- Week 3
- Week 6

**PIIANP**
- Week 4
- Week 6

**Notes**
- $n=12$, Mean ± SEM, *$p<0.05$, **$p<0.01$**
Regenerated cartilage:
SM04690 improved macroscopic cartilage appearance

- Rat knee samples 13 weeks after ACLT+pMMx
- Increased cartilage and smooth articular surface in SM04690 treated rats compared to vehicle
Summary: SM04690 is a promising DMOAD candidate

- Wnt signaling is a critical pathway in osteoarthritis
- In preclinical models, SM04690\(^{1-5}\)
  - Inhibited Wnt signaling
  - Inhibited protease production
  - Induced chondrogenesis
  - Inhibited inflammatory cytokine production
  - Demonstrated sustained local availability and no systemic exposure
  - Demonstrated no observable systemic toxicity
- Human clinical trials (Phase 2) are ongoing

Thank you

samumend