Anti-inflammatory properties of SM04690, a small molecule inhibitor of the Wnt pathway as a potential treatment for knee osteoarthritis

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Disclosures

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The Wnt pathway, osteoarthritis (OA), and inflammation

- Increased Wnt signaling drives bone formation, cartilage breakdown, and inflammation\textsuperscript{1-4}
- Wnt pathway mutations (e.g., FrzB, DOT1L) are associated with OA\textsuperscript{5,6}
- Wnt proteins are over-expressed in OA joints\textsuperscript{7,8}

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SM04690: A Wnt pathway inhibitor for knee OA

- SM04690 is a small molecule, intra-articular (IA), Wnt pathway inhibitor in development for treatment of knee OA\(^1,2\)
- In previous preclinical studies, SM04690:
  - inhibited inflammation\(^1\)
  - decreased cartilage degradation\(^1\)
  - regenerated cartilage\(^1\)
  - demonstrated sustained local exposure and no observable systemic toxicity\(^1,2\)
- In previous phase 1 and phase 2a clinical studies, a single IA SM04690 injection appeared well-tolerated and showed potential for improving symptoms and maintaining joint space width in knee OA subjects\(^3\)

The current studies evaluated SM04690 effects in an inflammatory model of OA.

1. Deshmukh V, et al. (2017) OAC.
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), where 0=weak activity and 5=highly potent activity.
- SM04690 demonstrated comparable or better activity than prednisolone and cyclosporin A 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>
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<tbody>
<tr>
<td></td>
<td>T Cell</td>
<td>B cell</td>
<td>Th17</td>
<td>Th1</td>
</tr>
<tr>
<td>SM04690 (37 nM)</td>
<td>5</td>
<td>3</td>
<td>3</td>
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<td>Cyclosporin A (120nM)</td>
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<td>Prednisolone (120nM)</td>
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</tbody>
</table>

Abbreviations: PBMC, peripheral blood mononuclear cells; HDF, human dermal fibroblasts; EC, endothelial cells; LPS, lipopolysaccharide
SM04690 anti-inflammatory activity - \textit{In vitro}
Decreased inflammation:
SM04690 suppressed inflammatory cytokines

Cellular assay:
• Synovial fibroblasts were stimulated with IL-1β to induce cytokine production, then treated with SM04690
• Cytokine production was quantified by ELISA and qRT-PCR
• Dose dependent inhibition of IL-1β, IL-6, IL-8, and TNF-α production was demonstrated

IL-6 $EC_{50} = 24\ \text{nM}$; TNF-α $EC_{50} = 35\ \text{nM}$

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

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

-Graphs showing normalized fold change for cytokines IL-1β, IL-2, IL-5, IL-6, IL-8, TNF-α, and IFN-γ. DMSO and SM04690 treatments are compared.

n=3 replicates, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001.
SM04690 inhibited LPS-stimulated inflammation in human monocytes via NFκB

**Cellular assay:**
- Human monocytes were stimulated with LPS and treated with SM04690 for 4hrs
- Levels of proteins were measured by Western blot
- SM04690 specifically inhibited NFκB phosphorylation *in vitro* and had no effects on other pathways
SM04690 anti-inflammatory activity - *In vivo*
Inflammatory model of rat OA: Monosodium Iodoacetate (MIA) injection

Rat MIA model:

Inflammation within 2 hours and cartilage degeneration within 1-2 weeks

- Monosodium iodoacetate (MIA) intra-articular (IA) injection on Day 0
- SM04690 IA injection on Day 3 (0.3 μg)
- Joint histology performed on Day 11 for histology and Day 28 for joint health
SM04690 attenuated acute inflammation in the rat MIA knee OA model compared to vehicle

- H&E staining after a single IA injection of SM04690 decreased inflammatory infiltrates, decreased hypercellularity, and improved structural integrity, compared to vehicle treatment at Day 11
- Synovial membrane thickness was significantly decreased in SM04690 joints compared to vehicle at Day 11

### H&E Staining

**Sham**

**MIA + Vehicle**

**MIA + SM04690 (0.3µg)**

### Synovial membrane thickness

- n=30 sections, Mean ± SEM, **p<0.01, one-way ANOVA**
SM04690 attenuated acute inflammation and protected cartilage in the rat MIA knee OA model

- A single IA injection of SM04690 decreased inflammatory cytokines and matrix metalloproteinases (MMPs), compared to vehicle treatment at Day 11

**Cytokine gene expression**

**Protease gene expression**

$n=10$ rats/group, Mean ± SEM, $^*p<0.05$, one-way ANOVA

$n=8$ rats/group, Mean ± SEM, $^*p<0.05$, $^{**}p<0.01$, t-test
SM04690 attenuated pain in the rat MIA knee OA model

- A single IA injection of SM04690 decreased pain (measured by Von Frey) and improved gait (measured as weight distribution), compared to vehicle treatment.
SM04690 protected cartilage in the rat MIA knee OA model

- A single IA injection of SM04690 improved Safranin O staining and OARSI scores compared to vehicle at Day 28

n= 10, Mean ± SD, *p<0.05, Mann-Whitney U test
Conclusions

From *in vitro* models:
- SM04690 demonstrated potent anti-inflammatory effects across a broad range of cytokines
- These effects appeared to be mediated via NFκB

In the MIA rat knee OA model, SM04690, compared to vehicle:
- Attenuated inflammation and structural damage to the knee
- Improved pain in treated rats
- Protected cartilage from catabolic breakdown
  (Model limitations included exaggerated inflammatory and degenerative responses compared to human knee OA.)

- Further studies elucidating the role of SM04690 in inflammatory pathways are on-going
- A human Phase 2b clinical trial is in progress
Thank you