Background

- Knee osteoarthritis (OA) is characterized by articular cartilage destruction, subchondral bone alterations, and synovitis.\(^1\)
- Wnt signaling affects OA pathogenesis by modulating inflammation, cartilage breakdown, and bone/cartilage formation. Increased Wnt signaling induces stem cell differentiation into osteoblasts while a decrease shifts lineage fate towards chondrogenesis.\(^2\)
- Samumed is developing a small molecule Wnt pathway inhibitor, SM04690, as a potential disease modifying OA drug.

Methods

- A small molecule library was screened using a cell-based TCF/LEF-luciferase reporter assay and hits were counter-screened using an SV-40-luciferase reporter. Expression of Wnt target genes (Axin2, Lef-1, TCF7, Cmyc, Axin2) were measured by qPCR in human mesenchymal stem cells (hMSCs).
- Global gene expression response to 16h treatment with SM04690 was measured in hMSCs using RNA-sequencing (RNA-seq).
- Chondrogenesis and osteogenesis were evaluated in hMSCs treated with SM04690 by immunocytochemistry and qPCR.
- Anti-inflammatory activity was evaluated by measuring TNF-α and IL-6 secretion using ELISA in IL-1β-stimulated synovial fibroblasts. Cytokine-induced GAG breakdown in chondrocytes was measured by DMMB assay.
- Pharmacokinetics & toxicology were evaluated in rats & dogs [single or multiple IA or IV injections (Q 30 days for up to 9 months)].
- In vivo activity of SM04690 was evaluated in a rat anterior cruciate ligament transection with partial meniscectomy (ACL+pmXm) model of OA. A single SM04690 (0.3 µg) IA injection was administered 1 week after transection.
- Week 5 after transection: Wnt pathway modulation in cartilage measured by a panel of 84 Wnt pathway genes using qPCR and β-catenin nuclear localization by immunohistochemistry (IHC). Protease and chondrogenic gene expression measured by qPCR.
- Week 13 after transection: Cartilage was evaluated using Osteoarthritis Research Society International (OARSI) histology scoring, and thickness measurements and Doublecortin (Dcx) positive chondrocytes were measured by IHC.
- Statistics - Parametric data: t-test for 2 groups, one-way ANOVA for >2 groups. Non-parametric data: Mann-Whitney U test.

Results

**SM04690 was a potent and specific inhibitor of Wnt signaling in vitro**

**SM04690 induced chondrocyte differentiation in hMSCs, demonstrated anti-inflammatory properties, and protected chondrocytes from catabolic breakdown in vivo**

**SM04690 regenerative cartilage in vivo in an ACLT+pmXm model of rat OA**

Conclusions

- SM04690 was a potent and specific inhibitor of canonical Wnt signaling.
- SM04690 induced chondrogenesis, inhibited cytokine production, and protected chondrocytes from catabolic breakdown.
- Following IA injection, SM04690 had prolonged residence time in the joint, no systemic exposure, and no systemic toxicity.
- Treatment with SM04690 inhibited Wnt signaling in vivo, increased cartilage thickness, increased chondrocyte numbers, and improved joint health in a rat injury model of knee OA.
- SM04690 has potential as a DMOAD. Human clinical trials are ongoing.

References