SM04690, a small molecule Wnt pathway inhibitor, induced chondrogenesis, protected MMP3 (n=10), and stimulated osteoblasts, as shown in qRT-PCR and Griess assay (n>3, Mean ± SEM, ***p<0.001). (c) Gene expression of chondrocyte markers in rat cartilage 4 weeks post treatment, measured by qRT-PCR. (d) Total sulfated GAG levels relative to tissue weight, measured by DMMB assay. (n=7 for vehicle and n=8 for treatment, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001).

**Methods**

- Wnt pathway inhibition was measured by qPCR in human meniscal chondrocyte cells (hMCCs).
- Chondrogenesis was evaluated using hMCCs by qRT-PCR and immunocytochemistry.
- Cytoplone induced protease release and glycosaminoglycan (GAG) breakdown in chondrocytes was measured by qRT-PCR and dimethylmethyelene blue (DMMB) assay.
- Anti-inflammatory activity was evaluated by measuring TNF-α and IL-6 secretion using ELISA in synovial fibroblasts stimulated with IL-1β. Pro-inflammatory cytokines (TNF-α, IL-1β, IL-2, IL-6, IL-17A, IL-17F, IFN-γ, & PGE2) were evaluated by ELISA in T and B cell co-cultures stimulated with superantigens or LPS, compared to vehicle or two benchmark immunosuppressants (cyclosporin A and prednisolone).
- Pharmacokinetics of SM04690 in plasma and joint were evaluated following intra-articular (IA) injection in rats.

In vivo activity of SM04690 was evaluated in a rat model: anterior cruciate ligament transection (ACLT+pmMx) using Osteoarthritis Research Society International (OARSI) scoring and biomarker measurement in knee and plasma by qPCR and ELISA.

**Results**

- SM04690 inhibited Wnt signaling and induced chondrocyte differentiation in hMCCs *in vitro*
  - **a** Wnt Target Genes
  - **b** Chondrogenic Genes
  - **c** Osteogenic Genes
  - **d** GAG (μg/mg)
  - **e** GAG (μg/mg)

**SM04690 protected chondrocyte from catabolic breakdown *in vitro***

- **a** Induce catabolism
  - IL-1β or TNFα + Oncostatin M
  - SM04690 or Control
  - Measure
  - MMP production/Secreted GAG and NO

- **b** Matrimetalloproteinases
  - MMP1
  - MMP3
  - MMP13
  - TNFα + OM
  - IL-1β

**SM04690 protected cartilage in the ACLT+pmMx model of rat OA**

- **a** OA RIS Score (Week 13)
  - Vehicle
  - SM04690 (0.3μg)
  - SM04690 (0.1μg)

**SM04690 inhibited inflammatory responses in co-culture systems *in vitro*** with comparable or greater potency than Cyclosporin A and Prednisolone

- **a** IL-6 EC50= 24.3μM, TNF-α EC50= 3.5μM
- **b** Unstimulated
  - L-1β (10μg/ml)
  - IL-1β + SM04690 (0.1μg)
  - IL-1β + SM04690 (0.3μg)

**Conclusions**

- SM04690, a small molecule Wnt pathway inhibitor, induced chondrogenesis, protected chondrocytes from catabolic breakdown, increased cartilage thickness and improved joint health in a rat model of knee OA.
- Additionally, potent anti-inflammatory effects of SM04690 observed in various cell types may provide beneficial effects in the treatment of OA.
- Human clinical trials with SM04690 are ongoing.

**References**


**Poster# 0503**

**Modifying Treatment for Knee Osteoarthritis**

**Wnt Pathway (SM04690) as a Potential Disease Signaling Pathogenesis**

**Background**

- Knee osteoarthritis (OA) is characterized by destruction of articular cartilage, subchondral bone alterations, and synovitis.1
- At a cellular level, Wnt signaling affects OA pathogenesis in joints by influencing inflammation, cartilage breakdown, and bone / cartilage formation. Increased Wnt signaling induces stem cells to differentiate into osteoblasts, and decreased signaling induces chondrogenesis.2
- Samumed is developing a small molecule Wnt pathway inhibitor, SM04690, as a potential disease modifying drug (DMOAD) injected into the knee.
- Preclinical studies of SM04690 were conducted to evaluate chondrogenesis, anti-inflammation, cartilage protection, and joint health.

**Methods**

- Wnt pathway inhibition was measured by qPCR in human meniscal chondrocyte cells (hMCCs).
- Chondrogenesis was evaluated using hMCCs by qRT-PCR and immunocytochemistry.
- Cytoplone induced protease release and glycosaminoglycan (GAG) breakdown in chondrocytes was measured by qRT-PCR and dimethylmethyelene blue (DMMB) assay.
- Anti-inflammatory activity was evaluated by measuring TNF-α and IL-6 secretion using ELISA in synovial fibroblasts stimulated with IL-1β. Pro-inflammatory cytokines (TNF-α, IL-1β, IL-2, IL-6, IL-17A, IL-17F, IFN-γ, & PGE2) were evaluated by ELISA in T and B cell co-cultures stimulated with superantigens or LPS, compared to vehicle or two benchmark immunosuppressants (cyclosporin A and prednisolone).
- Pharmacokinetics of SM04690 in plasma and joint were evaluated following intra-articular (IA) injection in rats.

In vivo activity of SM04690 was evaluated in a rat model: anterior cruciate ligament transection (ACLT+pmMx) using Osteoarthritis Research Society International (OARSI) scoring and biomarker measurement in knee and plasma by qPCR and ELISA.

**Results**

- SM04690 inhibited Wnt signaling and induced chondrocyte differentiation in hMCCs *in vitro*