

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

Presented at the Orthopaedic Research Society Annual Meeting, March 10-13, 2018,
New Orleans, LA.

Vishal Deshmukh¹, Timothy Seo¹, Maureen Ibanez¹, Sunil KC¹, Luis Dellamary¹, Charlene Barroga¹,
Yusuf Yazici¹
¹Samumed LLC, San Diego, CA

Introduction: Osteoarthritis (OA) is characterized by pain, deformity, and reduced function in the knee joint. Upregulated Wnt signaling affects the pathogenesis of OA through increased inflammation, increased subchondral bone and thinning cartilage. SM04690, a novel small molecule, was previously shown to exhibit OA disease modifying properties through inhibition of the Wnt pathway and induction of chondrogenesis *in vitro* and *in vivo*¹. SM04690 was evaluated in *in vitro* preclinical studies to determine its capacity to inhibit cytokine production and T and B cell proliferation and in an *in vivo* animal model to determine its capacity to reduce inflammation and cytokine production, protect cartilage, and reduce pain in OA.

Methods: Cytokine secretion (IL-6 and TNF- α) from synovial fibroblasts stimulated with IL-1 β was measured by ELISA to assess dose dependent anti-inflammatory activity and EC₅₀ of SM04690. The anti-inflammatory properties of SM04690 compared to vehicle or benchmark immunosuppressant (cyclosporine A) or steroid (prednisolone) were evaluated in (1) peripheral blood mononuclear cells (PBMCs) and (2) T and B cell co-cultures, both stimulated with either super-antigen (sAg) or Lipopolysaccharides (LPS). A panel of pro-inflammatory cytokines (TNF- α , IL-1 α , IL-1 β , IL-2, IL-6, IL-8, IL-17A, IL-17F, IFN- γ and PGE2) were measured by ELISA, and T and B cell proliferation by flow cytometry in both (1) and (2). Also, the effects of SM04690 on LPS-induced expression and phosphorylation of JNK, NF κ B, Erk, cJun, Akt, Stat3 in THP-1 cells were measured by qPCR and Western Blot. *In vivo* SM04690 activity was evaluated in a rat monosodium iodoacetate (MIA) injection-induced OA model, immediately followed by a single intra-articular SM04690 or vehicle injection. Joint inflammation was evaluated by measuring synovial thickness and infiltrating cells by histology; inflammatory cytokines (IL-1 α , IL-1 β , IL-2, IL-5, IL-6, IL-8, TNF- α and IFN- γ) by qPCR and ELISA; and cartilage protection by qPCR for matrix metalloproteinases (MMPs). Pain was measured as paw withdrawal threshold using Von Frey apparatus. Animal studies were approved by the Samumed, LLC Animal Committee and performed in accordance with the U.S. Department of Agriculture guidances. Statistical analyses used one-way ANOVA for multiple group comparisons and t-tests for comparison between two groups.

Results: SM04690 dose-dependently inhibited IL-1 β -induced TNF- α and IL-6 secretion in synovial fibroblasts (EC₅₀=30nM). In both (1) and (2), SM04690 significantly inhibited sAg- ($p<0.01$) and LPS- ($p<0.01$) stimulated pro-inflammatory cytokine production (Fig. A) as well as T and B cell proliferation ($p<0.01$), compared to DMSO treatment, with activity comparable to or better than cyclosporin A and prednisolone. SM04690 treatment specifically decreased LPS-induced gene expression ($p<0.01$) and phosphorylation of NF κ B in THP-1 cells, compared to DMSO, with no effects on JNK, Erk, cJun, Akt and Stat3. In the *in vivo* rat MIA OA model, compared to vehicle, SM04690 injection reduced inflammatory cells, decreased synovial thickness ($p<0.05$, Figure B), and inhibited production of proinflammatory cytokines and MMPs ($p<0.05$). SM04690 increased ($p<0.01$) paw withdrawal threshold in treated rats compared to vehicle at multiple time points (Figure C).

Discussion: SM04690 demonstrated potent anti-inflammatory properties in various cell types with activity comparable to or greater than benchmark agents, and inhibited NFkB signaling *in vitro*. In a rodent model of knee OA, a single IA injection of SM04690 resulted in reduced inflammation and pain compared to vehicle. Further studies to understand the mechanisms of Wnt signaling inhibition and anti-inflammatory effects are ongoing.

Significance: OA therapeutic options are currently limited to symptom relief. Disease modifying treatments are not currently approved for use in humans. With previously demonstrated regenerative effects in nonclinical studies¹, along with anti-inflammatory properties presented here, SM04690 shows potential as a single agent which may benefit symptoms and provide disease modification in OA.

References: 1. Hood JD, Deshmukh V, Barroga C, Hu Y. Discovery of a small molecule inhibitor of the Wnt pathway (SM04690) as a potential disease modifying treatment for knee osteoarthritis. *Osteoarthritis and Cartilage* 2016;24:S14 - S15.

Disclosures: Vishal Deshmukh, Timothy Seo, Maureen Ibanez, Sunil KC, Luis Dellamary, Charlene Barroga, Yusuf Yazici (Samumed, salary and equity)

