

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

Vishal Deshmukh, PhD¹, Charlene Barroga, PhD¹, Carine Bossard, PhD¹, Sunil KC, PhD¹, Tim Seo, MS¹, Melinda Pedraza, BS¹, Maureen Ibanez, MS¹, Kevin Chiu, BS¹, Long Do, PhD¹, Shawn Cho, MS¹, Luis Dellamary, BS¹, Josh Stewart, BS¹, Haide Hu, PhD², Betty Tam, PhD¹, John Hood, PhD², Yusuf Yazici, MD¹

¹Samumed, LLC

²Formerly Samumed, LLC

INTRODUCTION: Osteoarthritis (OA) is characterized by thinning cartilage and increased subchondral bone. Current therapeutic options focus on alleviating the symptoms and pain rather than disease modification. Amongst many cellular processes, Wnt signaling affects the pathogenesis of OA by influencing formation of bone and cartilage, cartilage breakdown, and inflammation in the joints. Wnt signaling is increased in the joints of OA patients and polymorphisms in genes involved in the Wnt pathway are associated with an increased susceptibility to development of OA. Increased Wnt signaling induces the expression of cartilage catabolic enzymes, as well as drives stem cells in the joint to differentiate into osteoblasts, while decreased Wnt signaling induces chondrogenesis. SM04690 was developed as a novel, small molecule inhibitor of the Wnt pathway, and evaluated in a series of preclinical studies to determine its capacity to induce chondrogenesis, protect cartilage, reduce inflammation and thereby improve joint health.

METHODS: Wnt pathway inhibition was measured using a cell-based luciferase reporter assay controlled by a beta-catenin/TCF-responsive promoter in SW480 colon cancer cells, an APC mutant cell line with constitutively active Wnt signaling. Expression of several downstream Wnt target genes (Ascl1, c-myc, Lef-1, Axin2, TCF4, TCF7, beta-catenin) were measured by qPCR and Western blot in human mesenchymal stem cells (hMSCs). Chondrogenic activity was evaluated by assessing differentiation of hMSCs to chondrocytes, using qPCR and immunocytochemistry. Cytokine induced matrix metalloproteinase (MMP) production and glycosaminoglycan (GAG) release from chondrocytes was measured by qRT-PCR and dimethylmethylene blue (DMMB) assays. Anti-inflammatory activity was evaluated by measuring TNF-alpha and IL-6 secretion using ELISA in synovial fibroblasts stimulated with IL1-beta or with lipopolysaccharides (LPS). Pharmacokinetics of SM04690 were evaluated by intra-articular (IA) injection in rats and dogs, followed by analysis of compound concentration in the joints and plasma; safety was assessed by clinical signs and histopathology. *In vivo* efficacy of SM04690 was evaluated in the rat instability model combining anterior cruciate ligament transection with medial meniscectomy. SM04690 was injected IA, followed by measurement of a panel of 84 Wnt pathway genes in the cartilage by qPCR, histological evaluation of cartilage using Osteoarthritis Research Society International (OARSI) scoring and biomarker measurement in the knee and plasma by qPCR and ELISA.

RESULTS: A small molecule screen using the beta-catenin/TCF-responsive promoter assay and iterative medicinal chemistry led to the development of SM04690, a potent ($EC_{50}=1\text{ nM}$) and selective inhibitor of Wnt signaling. SM04690 demonstrated 50-150-fold more potent activity as compared to several known Wnt pathway inhibitors (FH535, IWR1, KY02111, ICG001, iCRT14, CX4945). SM04690 (30nM) significantly inhibited expression of several downstream Wnt target genes (Ascl1, c-myc, Lef1, Axin2, TCF4, TCF7, beta-catenin) compared to controls, measured by qPCR (P less than 0.01) and Western Blot in hMSCs. SM04690 induced robust differentiation of hMSCs ($EC_{50}=30\text{ nM}$) into mature and functional chondrocytes as demonstrated by Sox9, Aggrecan, and Type II collagen expression, GAG production and positive staining for Alcian blue. SM04690 also inhibited cytokine induced Wnt pathway mediated protease (MMP1, MMP3, MMP13) production and GAG release from chondrocytes (P less than 0.01), compared to vehicle. Further, SM04690 inhibited IL1-beta and LPS induced TNF-alpha and IL6 secretion in synovial fibroblasts ($EC_{50}=30\text{ nM}$). *In vivo*, a single IA injection of SM04690 (0.3ug) in rats and dogs resulted in joint concentrations greater than EC_{50} for more than 180 days, with no detectable systemic exposure or toxicity up to more than 400X the expected clinical dose. This dose also inhibited the Wnt pathway *in vivo*, showing a decrease in several Wnt pathway genes (e.g. Dvl1, Axin2, TCF4, TCF7, cyclinD1) and a corresponding increase in Wnt pathway inhibitory genes (e.g. DKK1, SFRP1, WIF1). SM04690 treatment improved cartilage health, with histologically observed increased cartilage thickness, evidence for regeneration and protection from cartilage catabolism, resulting in significantly reduced OARSI score (P less than 0.01) and improved OA biomarkers (P less than 0.05) as compared to controls.

CONCLUSIONS: The Wnt pathway plays a critical role in the pathogenesis of OA. SM04690 was developed to be a potent and specific inhibitor of the Wnt pathway. SM04690 induced chondrogenesis with evidence for cartilage regeneration and inhibited cartilage breakdown both *in vitro* and in the rat OA model as compared to vehicle. Additionally, SM04690 reduced inflammation *in vitro*. A single IA injection of SM04690 resulted in long knee joint residence time, with no detectable systemic exposure or toxicity. SM04690 modulated key Wnt pathway driven processes of cartilage regeneration and degradation, and therefore has potential as a disease modifying therapy for OA. Phase II human clinical studies are ongoing.