Title

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

Authors: John Hood, Vishal Deshmukh, Charlene Barroga, Yong Hu

Background/Purpose:

Osteoarthritis (OA) is characterized by increased subchondral bone and thinning cartilage. The Wnt signaling pathway has the capacity to regulate both of these processes. Increased Wnt signaling induces stem cells in the joint to become osteoblasts while decreased Wnt signaling induces chondrogenesis. Wnt signaling is increased in the joints of OA patients and polymorphisms in genes involved in Wnt signaling are associated with an increased susceptibility to development of OA. SM04690, a novel, small molecule inhibitor of the Wnt pathway, was evaluated in a series of preclinical studies to determine its capacity to induce chondrogenesis and improve joint health.

Methods:

The capacity of SM04690 to inhibit the Wnt pathway was determined with a cellular screen utilizing a luciferase reporter controlled by a Wnt-responsive promoter. The ability of SM04690 to induce chondrogenesis was evaluated using primary human mesenchymal stem cells (MSCs); differentiation of MSCs to chondrocytes was determined by gene expression analysis and staining for chondrogenic markers. The capacity of SM04690 to block protease release from human chondrocytes was assessed by treating chondrocytes with TNFα and Oncostatin M and measured by ELISA. The effect of SM04690 on TNFα and IL-6 production by synovial fibroblasts was measured by inducing cytokine release with IL1β followed by measurement by ELISA. Pharmacokinetics of SM04690 were evaluated by intra-articular (IA) injection in Sprague Dawley rats and beagle dogs followed by analysis of compound concentration in the joints and plasma. Safety of SM04690 was assessed by IA injection in Sprague Dawley rats and beagle dogs followed by evaluation of clinical signs and histology. *In vivo* activity of SM04690 was evaluated in the rat instability model combining anterior cruciate ligament transection with medial meniscal tear. SM04690 was injected into the IA space of the damaged knee, followed by histological evaluation using OARSI scoring and measurement of biomarkers from plasma by ELISA.

Results:

In vitro, SM04690 inhibited Wnt pathway activation with an $EC_{50} \cong 3$ nM. Consistent with Wnt's role in chondrogenesis, SM04690 induced MSCs to differentiate into chondrocytes with an $EC_{50} \cong 30$ nM (**Figure 1**). SM04690 inhibited production of proteases from human chondrocytes suggesting it has the capacity to block a key mediator of cartilage degradation in OA. SM04690 blocked IL1β-induced production of TNFα and IL-6, suggesting it may reduce the inflammatory component of OA. *In vivo*, a single IA injection of SM04690 into Sprague Dawley rats or Beagle dogs resulted in an IA concentration above the target EC_{50} with no detectable systemic

exposure. No systemic toxicity was observed after single or monthly IA injections of doses >1400X the therapeutic dose (6 or 9 monthly injections for rats or dogs, respectively). In the rat instability model, a single IA injection of SM04690 2 weeks post-injury improved cartilage health in a dose-dependent manner relative to vehicle control. Representative histology in the rat instability model showed increased cartilage thickness in SM04690-treated animals relative to vehicle control (**Figure 2**). At the optimal IA dose in rats (0.3 μg/knee) OARSI scores decreased significantly, p=0.006.

Conclusion:

SM04690 has been shown in these experiments to inhibit the Wnt pathway, induce chondrogenesis, inhibit protease production and improve cartilage health in rodent models of OA after a single IA injection. SM04690 was maintained in the joint space with no detectable exposure in the plasma, indicative of a low potential for systemic toxicity. These data suggest that locally injected SM04690 has potential as a disease modifying therapy for OA.

Figure 1. SM04690 induces chondrogenesis in human mesenchymal stem cells

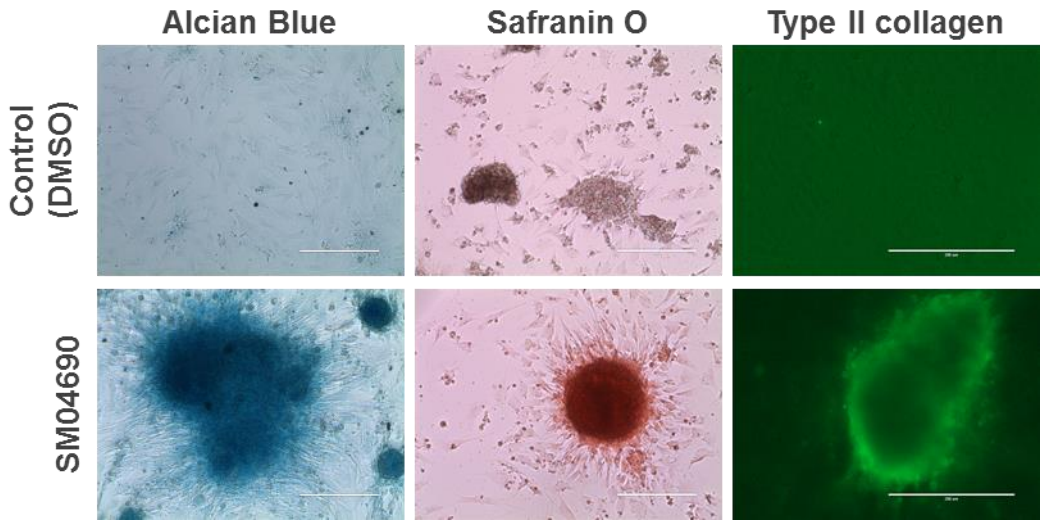

Figure 2. SM04690 increases cartilage thickness relative to control in rat instability model
arrows designate articular cartilage