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

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Disclosures: Vishal Deshmukh, Charlene Barroga, Maureen Ibanez, Luis Dellamary, Josh Stewart, Yusuf Yazici (Samumed, LLC salary and equity), John Hood and Haide Hu (Samumed, LLC, equity)

ABSTRACT INTRODUCTION: Osteoarthritis (OA) is characterized by increased subchondral bone and thinning cartilage. Current therapeutic options focus on alleviating the symptoms and pain rather than disease modification. Stem cell and growth factor based treatments are under investigation, but have not definitively established either safety or efficacy. Amongst many cellular processes, Wnt signaling affects the pathogenesis of OA by influencing inflammation, cartilage breakdown, and formation of bone and cartilage in joints. Increased Wnt signaling induces stem cells in the joint to differentiate into osteoblasts, while decreased Wnt signaling induces chondrogenesis. 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. 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, reduce inflammation, protect cartilage, and thereby improve joint health.

METHODS: Wnt pathway inhibition was measured using a cell-based luciferase reporter assay controlled by a Wnt-responsive promoter. Chondrogenesis was evaluated using differentiation of human mesenchymal stem cells (hMSCs) to chondrocytes by qRT-PCR and immunocytochemistry. Cytokine induced protease release and glycosaminoglycan (GAG) breakdown in chondrocytes was measured by qRT-PCR and dimethylmethylene blue (DMMB) assay. Anti-inflammatory activity was evaluated by measuring TNFα and IL6 secretion using ELISA in synovial fibroblasts stimulated with IL1β and THP1 monocytes stimulated with lipopolysaccharides (LPS). A panel of pro- and anti-inflammatory cytokines (TNFα, IL1α, IL1β, IL2, IL6, IL8, IL17A, IL17F, IFNγ, and PGE2) was evaluated by ELISA, T and B cell proliferation were measured by flow cytometry in PBMCs, and T and B cell co-cultures were stimulated with superantigen or LPS, compared to vehicle or two benchmark immunosuppressants (cyclosporin A and prednisolone). 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 following IA injection by clinical signs and histopathology. *In vivo* activity of SM04690 was evaluated in the rat instability model combining anterior cruciate ligament transection with medial meniscal tear. SM04690 was injected IA, followed by histological evaluation using Osteoarthritis Research Society International (OARSI) scoring and biomarker measurement in the knee and plasma by qPCR and ELISA.

RESULTS SECTION: SM04690 demonstrated potent (EC_{50} ≅11nM) and selective inhibition of Wnt signaling. *In vitro,* SM04690 induced robust differentiation of hMSCs (EC_{50}≅30nM) into mature and functional chondrocytes (Figure A), as well as inhibited cytokine induced protease release (Figure B) and GAG breakdown from chondrocytes (P<0.01) compared to vehicle treatment. SM04690 inhibited IL1β and LPS induced TNFα and IL6 secretion in synovial fibroblasts and THP1 monocytes, respectively (EC_{50}≅30nM). SM04690 significantly inhibited (P<0.01, effect size >40%, no toxicity) superantigen and LPS stimulated production of the pro-inflammatory panel of cytokines, and T and B cell proliferation in PBMCs and T and B cell co-cultures (Figure C), with activity comparable to or better than the activities of cyclosporin A and prednisolone. *In vivo*, a single IA injection of SM04690 (0.3µg) in rats and dogs resulted in joint concentrations >EC50 for >180 days, with no detectable systemic exposure or toxicity up to >400X the expected clinical dose. This dose also inhibited the Wnt pathway *in vivo*, showing a decrease in Axin2 and beta catenin and a corresponding improvement in cartilage health with histologically observed increased cartilage thickness (Figure D), evidence for regeneration and protection from cartilage catabolism, resulting in significantly reduced OARSI score (P<0.01) (Figure E) and OA biomarkers (P<0.05) as compared to vehicle.

DISCUSSION: SM04690 was shown to inhibit the Wnt pathway and induce chondrogenesis *in vitro* and *in vivo.* Additionally, SM04690 reduced inflammation *in vitro* and inhibited the production of proteases both *in vitro* and in the rat model, suggesting it has the capacity to inhibit key mediators of cartilage degradation in OA. In a rodent model of knee OA, a single IA injection of SM04690 resulted in long local residence time, reduced protease expression, evidence for cartilage regeneration and improved cartilage thickness and health, compared to vehicle, with no detectable exposure in the plasma or systemic toxicity. SM04690 has potential as a disease modifying therapy for OA.

SIGNIFICANCE: OA currently has limited therapeutic options that focus on alleviating pain until knee replacement. Disease modifying treatments are not currently approved for use in humans. A single IA injection of a small molecule Wnt inhibitor, SM04690, demonstrated reduced inflammation, increased cartilage protection, and cartilage regeneration in a rodent model of OA with no observed toxicity. These data suggest SM04690 may be an effective disease modifying treatment for OA. Clinical studies are ongoing.

IMAGES AND TABLES: