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Modulation of the Wnt Pathway through Inhibition of CLK2 and DYRK1A by SM04690, a Novel Potential Disease-Modifying Treatment for Knee Osteoarthritis

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Objectives: Wnt pathway upregulation contributes to osteoarthritis (OA) through differentiation of stem cells into osteoblasts, increased catabolic enzymes, and inflammation. SM04690, a novel small-molecule Wnt pathway inhibitor, previously demonstrated chondrogenesis and cartilage protection. SM04690 was evaluated in preclinical studies to determine its mechanism of action for Wnt pathway inhibition, chondrogenesis, and anti-inflammatory effects.

Design: Kinase activity was measured using Z-lyte and Lantha assays. Protein phosphorylation in human mesenchymal stem cells (hMSCs), chondrocytes, and synovial fibroblasts was measured by Western blot. Expression of Wnt pathway and chondrogenic genes and LPS-induced inflammatory cytokines were measured in siRNA knockdowns in hMSCs and BEAS-2B cells by qPCR. *In vivo*, effects of SM04690 on inflammation, pain, and function were evaluated in rat OA models, followed by single intra-articular (IA) injection of SM04690 or vehicle.

Results: SM04690 primarily inhibited intranuclear kinases cdc-like kinase 2 (CLK2, EC₅₀: 5.8 nM) and dual-specificity tyrosine kinase (DYRK1A, EC₅₀: 26.9 nM). SM04690 inhibited CLK2-mediated phosphorylation of alternative splicing regulators, serine and arginine rich (SR) proteins, and DYRK1-mediated phosphorylation of Sirt1 and FoxO1. siRNA knockdowns identified roles for 1) CLK2 and DYRK1A in Wnt pathway modulation with no effects on β -catenin and 2) CLK2 inhibition in early chondrogenesis with DYRK1A inhibition playing a role in enhancing late chondrocyte function. NF κ B and STAT3 inhibition by SM04690 resulted in reduced inflammatory cytokines compared to controls. DYRK1A knockdown was sufficient, while combined DYRK1A/CLK2 knockdown enhanced DYRK1A knockdown anti-inflammatory effects. *In vivo* models showed that SM04690 inhibited inflammatory cytokine production and expression of cartilage degradative enzymes, resulting in increased joint cartilage, decreased pain, and improved function.

Conclusions: Inhibition of CLK2 and DYRK1A by SM04690 demonstrated a novel dual mechanism for inhibiting the Wnt pathway and enhancing chondrogenesis, chondrocyte function, and anti-inflammation in rat models of knee OA. SM04690 shows potential as an agent which may improve structure, symptoms, and function of OA.