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## **Wnt Pathway Modulation via CLK2 and DYRK1A Inhibition by Lorecivivint, a Potential Disease-Modifying Treatment for Knee Osteoarthritis**

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**Background:** Wnt pathway upregulation contributes to osteoarthritis (OA) through increased osteoblast differentiation and increased catabolic enzyme/inflammatory cytokine levels. Lorecivivint (SM04690), a novel, small-molecule Wnt pathway inhibitor, previously demonstrated preclinical chondrogenesis and cartilage protection. Lorecivivint was evaluated in preclinical studies to determine its mechanism of action for Wnt pathway inhibition, chondrogenesis, and anti-inflammatory effects.

**Methods:** Kinase activity was measured using Z-LYTE and Lantha assays. Protein phosphorylation in human mesenchymal stem cells (hMSCs), chondrocytes, and synovial fibroblasts were measured by Western blot. Wnt pathway/chondrogenic genes and LPS-induced inflammatory cytokines were measured by qPCR in siRNA knockdowns (hMSCs/BEAS-2B cells). *In vivo* lorecivivint effects on inflammation, pain, and function were evaluated in rat OA models compared to vehicle.

**Results:** Lorecivivint inhibited intranuclear kinases CDC-like kinase 2 (CLK2, EC<sub>50</sub>: 5.8 nM) and dual-specificity tyrosine kinase (DYRK1A, EC<sub>50</sub>: 26.9 nM). Lorecivivint inhibited CLK2-mediated phosphorylation of serine and arginine rich splicing factor proteins and DYRK1A-mediated phosphorylation of Sirt1 and FoxO1. siRNA knockdowns identified roles for 1) CLK2 and DYRK1A in Wnt pathway modulation with no effects on  $\beta$ -catenin and 2) CLK2 inhibition in early chondrogenesis with DYRK1A inhibition playing a role in enhancing late chondrocyte function. NFKB/STAT3 inhibition by lorecivivint resulted in reduced inflammatory cytokine gene expression compared to controls. DYRK1A knockdown inhibited inflammation, and this effect was enhanced with combined DYRK1A/CLK2 knockdown. *In vivo* models showed that lorecivivint inhibited inflammatory cytokine production and expression of cartilage degradative enzymes, resulting in increased joint cartilage, decreased pain, and improved function.

**Conclusion:** Lorecivivint demonstrated a novel dual mechanism of action for Wnt pathway inhibition via CLK2 and DYRK1A, enhanced chondrogenesis and chondrocyte function, and reduced inflammation in rat models of knee OA (Figure). Lorecivivint may improve the structure, symptoms, and function of knee OA.

