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## **Inhibition of CLK2 and DYRK1A by Lorecivivint (SM04690) as a Novel Molecular Regulator of Wnt Signaling, Chondrogenesis, and Inflammation, a Potential Disease-Modifying Treatment for Knee Osteoarthritis**

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**Background:** In the synovial joint, Wnt pathway upregulation contributes to osteoarthritis (OA) by increasing osteocyte differentiation, cartilage thinning, and inflammation. SM04690, a novel small molecule, has previously demonstrated potential OA disease-modifying effects through Wnt pathway inhibition *in vitro* and *in vivo*.

**Objective:** To elucidate the novel mechanism of action for SM04690 on Wnt pathway inhibition, chondrocyte differentiation, and anti-inflammation.

**Methods:** Wnt pathway activity was measured using a cell-based TCF/LEF luciferase reporter in SW480 colon cancer cells. A kinome screen (318 kinases) was performed. The effects of SM04690 on protein phosphorylation of serine and arginine rich splicing factors (SRSF proteins), Sirt1, and FoxO1 in hMSCs, chondrocytes, and synovial fibroblasts were measured by Western blot. The effects of SM04690 and siRNA knockdown (KD) on chondrogenic and Wnt pathway gene expression were measured by NanoString gene expression panels and effects on LPS-induced inflammatory cytokines (IL-6, IL-8, TNF- $\alpha$ ) in BEAS-2B cells were measured by qPCR and ELISA. *In vivo*, the pharmacodynamic effects of SM04690 were evaluated in monosodium iodoacetate injection-induced and anterior cruciate ligament transection with partial medial meniscectomy rat knee OA models in which a single intra-articular SM04690 (0.1  $\mu$ g, 0.3  $\mu$ g, 1.0  $\mu$ g) or vehicle injection was administered. Cartilage was isolated at Day 10 and 35; phosphorylation and expression of SRSF proteins, Sirt1, FoxO1, STAT3, and NF- $\kappa$ B were measured by Western blot.

**Results:** SM04690 was a potent ( $EC_{50}=11$ nM) inhibitor of Wnt signaling. CDC-like kinases (CLKs) and dual-specificity tyrosine kinase (DYRK1A) were identified as molecular targets of SM04690. In hMSCs and chondrocytes, compared to DMSO, SM04690 potently inhibited CLK-mediated phosphorylation of SRSF proteins. SM04690 also inhibited DYRK1A-mediated Sirt1 and FoxO1 phosphorylation, thus increasing total and nuclear FoxO1 levels. Compared to siRNA control, DYRK1A/CLK2 dual KD increased expression of chondrogenic genes (*COL2A1*, *ACAN*, *COMP*, *CD44* [all  $P<0.05$ ]). CLK2 and DYRK1A KDs each inhibited Wnt pathway genes (*AXIN2*, *TCF7*, *TCF4*, *LRP5*, *FZD6*, *FZD7*, *PITX2* [all  $P<0.05$ ]) with no effects on  $\beta$ -catenin levels, compared to siRNA control. In synovial fibroblasts, compared to DMSO, SM04690 decreased phosphorylation of NF- $\kappa$ B and STAT3. In BEAS-2B cells, compared to siRNA control, DYRK1A

KD inhibited inflammatory cytokine production (IL-6, IL-8, TNF- $\alpha$  [all  $P < 0.05$ ]), while DYRK1A/CLK2 dual KD enhanced anti-inflammatory effects of DYRK1A KD. In cartilage from rat OA models, compared to vehicle, SM04690 inhibited phosphorylation of SRSF proteins, Sirt1, FoxO1, and STAT3 as well as expression of NF- $\kappa$ B.

**Conclusion:** To our knowledge, this is the first report of SM04690 inhibition of nuclear kinases CLK2 and DYRK1A leading to effects on the Wnt pathway, chondrocytes, and inflammation (**Image**). This dual mechanism of SM04690 potentially modifies OA through increased chondrocyte differentiation and function and benefits symptoms through anti-inflammatory activity. Human trials are ongoing.

**Image:** Schematic representation of SM04690's proposed mechanism of action via CLK2 and DYRK1A inhibition in OA

