**Lorecivivint (SM04690), a Potential Disease-Modifying Osteoarthritis Drug, Inhibits CLK2 and DYRK1A, Novel Molecular Regulators of Wnt Signaling, Chondrogenesis, and Inflammation**

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**Background**

- Upregulated Wnt signaling affects osteoarthritis (OA) pathogenesis by increasing inflammation, subchondral bone formation, and thinning cartilage
- Lorecivivint (LOR), a novel small molecule, has demonstrated OA disease-modifying properties through Wnt pathway inhibition *in vitro* and *in vivo*.
- The mechanism of action of LOR leading to Wnt pathway inhibition, chondrocyte differentiation, and anti-inflammatory activity is described

**Methods**

**In vitro**

- Wnt pathway inhibition was assessed by luciferase reporter assay in SW480 colon cancer cells
- A kinome screen (318 kinases) was performed
- LOR effects on phosphorylation of proteins in human mesenchymal stem cells (hMSCs), chondrocytes, 293T cells, and synovial fibroblasts were measured by Western blot
- LOR effects on splicing were measured in hMSCs by RNA sequencing and PCR
- LOR and siRNA knockdown effects on hMSC Wnt pathway and chondrogenic gene expression were measured using nCounter® panels and qPCR

**In vivo**

- LOR effects were confirmed in rat knee OA models: (1) *surgical*; anterior cruciate ligament transection with partial medial meniscectomy (ACLT+pMMX) and (2) inflammatory: monosodium iodoacetate injection-induced knee OA model (data not shown)

**Statistical analyses:** One-way ANOVA (multiple groups) and t-tests (two groups)

**Results**

**LOR: A potent inhibitor of the Wnt pathway, CLK2, and DYRK1A in vitro**

- Luciferase reporter assay identified LOR as an inhibitor of Wnt signaling (IC_{50} = 11 nM). A kinome screen identified CDC-like kinases (CLK2, IC_{50} = 5.8 nM) and dual-specificity tyrosine kinase (DYRK1A, IC_{50} = 26.9 nM) as molecular targets of LOR

**Figure 1.** LOR treatment of hMSCs and chondrocytes resulted in decreased phosphorylation of SRSF4, Sirt1, and FoxO1 compared to DMSO

**Figure 2.** LOR modulated alternative splicing *in vitro*

**Figure 3.** LOR modulated the Wnt pathway independently of β-catenin

**Figure 4.** Inhibition of CLK2 and DYRK1A reduced Wnt pathway gene expression

**Figure 5.** Inhibition of CLK2/DYRK1A induced chondrocyte differentiation

**Figure 6.** LOR reduced inflammation via inhibition of CLK2 and DYRK1A

**Conclusions**

**In vitro and in vivo**

- LOR inhibited intranuclear kinases CLK2 & DYRK1A, leading to Wnt pathway inhibition
- Inhibition of CLK2 induced early chondrocyte differentiation from hMSCs and inhibition of DYRK1A enhanced chondrocyte function
- Inhibition of STAT3 phosphorylation and NF-κB expression by LOR provided potent anti-inflammatory effects
- Through dual inhibition of CLK2 and DYRK1A, LOR protected cartilage, induced chondrogenesis, and reduced inflammation, supporting its potential for modifying disease and improving signs and symptoms in knee OA

**Statistical analyses:** One-way ANOVA (multiple groups) and t-tests (two groups)