Lorecivivint (SM04690), a potential disease-modifying treatment for knee osteoarthritis, functions through inhibition of CLK2 and DYRK1A, novel molecular regulators of Wnt signaling, chondrogenesis, and inflammation

Vishal Deshmukh, PhD

All authors are employees or shareholders of Samumed, LLC
Osteoarthritis (OA) and the Wnt pathway

Degenerative tissue remodeling is due to mechanical forces and inflammation\textsuperscript{1}

Overexpressed Wnt proteins and pathway mutations are associated with OA\textsuperscript{2-5}

Increased Wnt signaling drives bone formation, cartilage breakdown, and inflammation\textsuperscript{6-9}

Hypothesis: Inhibiting the Wnt pathway reduces inflammation while protecting and regenerating cartilage
Lorecivivint (LOR; SM04690) preclinical development

In vitro assays and animal models of OA

hMSC assays

Protease assays

Cartilage Protection

Cytokine assays

Anti-inflammation

Animal models

Chondrocyte Regeneration

Cartilage Protection

Protease gene expression

Cytokine gene expression

Safranin O

Alcian blue

Type II collagen

Control

(SMSO)

SM04690

(30 nM)

Protease gene expression

Cytokine gene expression

Control Knee

13 weeks

SM04690-Treated Knee (0.3 μg)

13 weeks

Improved Joint Health

(Animal models)
Lorecivivint inhibits the Wnt pathway through a unique MOA.
Lorecivivint is a potent and selective kinase inhibitor

318 kinases tested in vitro

<table>
<thead>
<tr>
<th>Kinase Tested</th>
<th>% Inhibition Lorecivivint (0.5 µM)</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
<th>Fold IC&lt;sub&gt;50&lt;/sub&gt; &gt;CLK2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLK2</td>
<td>98</td>
<td>5.8</td>
<td>1.0</td>
</tr>
<tr>
<td>CLK3</td>
<td>100</td>
<td>44.3</td>
<td>7.6</td>
</tr>
<tr>
<td>DYRK1A</td>
<td>99</td>
<td>26.9</td>
<td>4.6</td>
</tr>
<tr>
<td>DYRK1B</td>
<td>94</td>
<td>41.2</td>
<td>7.1</td>
</tr>
<tr>
<td>GSK3B</td>
<td>92</td>
<td>37.8</td>
<td>6.5</td>
</tr>
<tr>
<td>HIPK1</td>
<td>95</td>
<td>33.2</td>
<td>5.7</td>
</tr>
<tr>
<td>HIPK2</td>
<td>95</td>
<td>16.8</td>
<td>2.9</td>
</tr>
</tbody>
</table>

- DNA
- Transcription
  - DYRK1A

- Pre-mRNA

- mRNA processing
e.g. splicing

- mRNA

- Translation

- Protein
Alternative splicing regulation of gene expression

DNA → Transcription → Pre-mRNA → mRNA processing e.g. splicing → mRNA → Translation → Protein

alternative splicing

Transcription

mRNA processing e.g. splicing

Translation

Protein A

Protein B

Protein C

Exon 1
Exon 2
Exon 3
Exon 4
Exon 5

DNA
Pre-mRNA
mRNA
Translation
DNA
Exon 1
Exon 2
Exon 3
Exon 4
Exon 5

Alternative Splicing

Intron retention
CDC-like kinases (CLKs)

Directly affects transcription or alternative splicing of genes

Alteration of transcription factors can subsequently impact target genes of implicated pathway

Splicing modes

Functional consequences of alternative splicing

- Altered mRNA stability
- Differential translation
- Modulation of protein activities (e.g., alt. exons coupled with alt. polyadenylation in MNK2)
- Dominant gain of functions (e.g., exon skipping in S6K1)
- Partial loss of functions (e.g., exon inclusion in BIN1)
- Proteins with opposite functions

Lorecivivint inhibited CLK-mediated SRSF phosphorylation

**Lorecivivint**

(*In vitro* CLK2 biochemical kinase assay)

- **Log Conc. - Lorecivivint (nM)**
- **% Inhibition**

- CLK2
- DMSO

**CLK2 IC\textsubscript{50} = 7.8 nM**

**SRSF**

(*hMSCs in vitro*)

- MW
- pSRSF4
- pSRSF6
- pSRSF5

- β-actin
Lorecivivint induced intron retention and modulated alternative splicing \textit{in vitro}

RNA sequencing in hMSCs
Lorecivivint inhibited DYRK1A

- DYRK1A inhibition
  - Reduced Wnt signaling\(^1\) (benefited chondrocytes)
  - Reduced SIRT1\(^1,2\) and increased FOXO1\(^3,4\) (benefited chondrocytes)
  - Reduced STAT3\(^5\) (inhibited inflammation)

TF: Transcription factor
HD: Histone deacetylase

\(\text{DMSO} \quad \text{DYRK1A} \quad \text{Lorecivivint (In vitro DYRK1A biochemical kinase assay)}\)

DYRK1A IC\(50\) = 26.9 nM

Lorecivivint inhibited SIRT1 and FOXO1 phosphorylation
Reduced FOXO1 phosphorylation led to increased nuclear FOXO1 levels

**SIRT1**
(hMSCs *in vitro*)

<table>
<thead>
<tr>
<th>IL1-β (20 ng/ml)</th>
<th>Unstimulated</th>
<th>DMSO</th>
<th>Lorecivivint (100 nM)</th>
<th>Lorecivivint (30 nM)</th>
<th>Lorecivivint (10 nM)</th>
<th>Lorecivivint (3 nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pSirt1 (Ser27)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pSirt1 (Ser47)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Sirt1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**FOXO1**
(Chondrocytes *in vitro*)

<table>
<thead>
<tr>
<th>IL1-β (20 ng/ml)</th>
<th>Unstimulated</th>
<th>DMSO</th>
<th>Lorecivivint (100 nM)</th>
<th>Lorecivivint (30 nM)</th>
<th>Lorecivivint (10 nM)</th>
<th>Lorecivivint (3 nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pSirt1 (Ser47)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total FoxO1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DAPI / FoxO1
CLK2 and DYRK1A knockdowns inhibited the Wnt pathway

- Knockdowns inhibited Wnt pathway genes and upregulated secreted Wnt inhibitors SFRP2 and DACT1

*In vitro* siRNA knockdown effects in hMSCs identified by Nanostring panel and validated by qPCR

*p<0.05, **p<0.01, ***p<0.001 vs. siCtrl*
Combined CLK2 / DYRK1A knockdown induced chondrocyte differentiation

*In vitro* siRNA knockdown effects in hMSCs identified by Nanostring panel and validated by qPCR

*p<0.05, **p<0.01, ***p<0.001 vs. siCtrl*
Lorecivivint decreased phosphorylation of NF-κB and STAT3

<table>
<thead>
<tr>
<th>NF-κB and STAT3</th>
<th>In vitro LPS-stimulated synovial fibroblasts</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Unstimulated</th>
<th>LPS + DMSO</th>
<th>LPS + Lorecivivint</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 nM</td>
<td>30 nM</td>
<td>10 nM</td>
</tr>
<tr>
<td>pNF-κB (p105)</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>Total NF-κB (p105/p50)</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>pStat3 (S727)</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>pStat3 (Y705)</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>Total Stat3</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>β-actin</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
</tbody>
</table>
Inhibition of CLK2 and DYRK1A inhibited inflammation

In vitro siRNA knockdown effects in BEAS2B cells
Cytokines measured by qPCR
*p<0.05, **p<0.01, ***p<0.001 vs. vehicle
Lorecivivint mechanism of action

Osteoarthritis

Wnt/Mechanical stress/Metabolic/Trauma

Structural Damage

Wnt gene expression

- TCF7
- NF-κB

Alt. splicing

Lorecivivint

CLK2

FOXO1

Chondrocyte differentiation/function

DYRK1A

Inflammatory gene expression

Symptoms

Inflammatory cytokines

- SIRT1
- STAT3

+ Altered protein levels

+ STAT3: signal transducer and activator of transcription 3, SIRT1: sirtuin 1, TCF7: transcription factor 7, NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells, FOXO1: forkhead Box O1
Lorecivivint summary

• The intranuclear kinases CLK2 and DYRK1A, dual targets of lorecivivint, are novel targets for modulation of Wnt signaling, chondrocyte biology, and inflammation
• Lorecivivint protected cartilage, induced chondrogenesis, and reduced inflammation *in vitro* and *in vivo*
• Phase 3 human clinical trials are ongoing
Thank you