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
DISCLOSURES

All authors are employees or shareholders of Samumed, LLC
Degenerative tissue remodeling is due to mechanical forces and inflammation\(^1\)

Overexpressed Wnt proteins and pathway mutations are associated with OA\(^2\)\(^-\)\(^5\)

Increased Wnt signaling drives bone formation, cartilage breakdown, and inflammation\(^6\)\(^-\)\(^9\)

Hypothesis: Inhibiting the Wnt pathway reduces inflammation while protecting and regenerating cartilage

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**Osteoarthritis (OA) and the Wnt pathway**

- Bone remodeling
- Bone sclerosis
- Osteophyte formation
- Cartilage degradation
- Chondrocyte hypertrophy

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**Figure adaptation:** Bush and Beier. Nature Medicine. 2013

Lorecivivint (LOR; SM04690) preclinical development

**In vitro assays and animal models of OA**

- **hMSC assays**
- **Protease assays**
- **Cytokine assays**
- **Animal models**

**Chondrocyte Regeneration**

**Cartilage Protection**

- **Protease gene expression**
  - MMP1
  - MMP3
  - MMP13
  - ADAMTS5

- Relative Expression

- Control (SM04690 (300μM))

**Anti-inflammation**

- **Cytokine gene expression**
  - IL-1β
  - TNF-α
  - IL-6

- Relative Expression

- Control Knee 13 weeks
- SM04690-Treated Knee (0.3 μg) 13 weeks

**Sustained Local PK**

- Expected therapeutic level (~30 nM)

**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_{50} (nM)</th>
<th>Fold IC_{50} &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 ← CLK2

mRNA

Translation

Protein
Alternative splicing regulation of gene expression

DNA

↓

Transcription

Pre-mRNA

↓

mRNA processing e.g. splicing

mRNA

↓

Translation

Protein
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

1. Altered mRNA stability
2. Differential translation
3. Modulation of protein activities (e.g., altered exons coupled with altered polyadenylation in MNK2)
4. Dominant gain of functions (e.g., exon skipping in SGK1)
5. Partial loss of functions (e.g., exon inclusion in BIN1)
6. Proteins with opposite functions

Lorecivivint inhibited CLK-mediated SRSF phosphorylation

**Lorecivivint**

(In vitro CLK2 biochemical kinase assay)

- % Inhibition vs. Log Conc. - Lorecivivint (nM)
- CLK2 IC\textsubscript{50} = 7.8 nM

**SRSF**

(hMSCs in vitro)

- MW Ladder DMSO 100 nM 30 nM 10 nM 3 nM
- pSRSF4 pSRSF6 pSRSF5
- β-actin
Lorecivivint induced intron retention and modulated alternative splicing 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)

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

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

<table>
<thead>
<tr>
<th></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>pSirt1 (Ser47)</td>
<td>pFoxO1</td>
<td>Total FoxO1</td>
<td>β-actin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pSirt1 (Ser27)</td>
<td>pSirt1 (Ser47)</td>
<td>pFoxO1</td>
<td>Total FoxO1</td>
<td>β-actin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
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<tr>
<th></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>DAPI / FoxO1</td>
<td>DAPI / FoxO1</td>
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<td>DAPI / FoxO1</td>
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</tbody>
</table>

IL1-β (20 ng/ml)
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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Relative Expression</th>
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<tbody>
<tr>
<td>COL2A1</td>
<td></td>
</tr>
<tr>
<td>CD44</td>
<td></td>
</tr>
<tr>
<td>RUNX1</td>
<td></td>
</tr>
<tr>
<td>PRG4</td>
<td></td>
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</tbody>
</table>

**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

**NF-κB and STAT3**

*In vitro* LPS-stimulated synovial fibroblasts

<table>
<thead>
<tr>
<th></th>
<th>Unstimulated</th>
<th>LPS + DMSO</th>
<th>LPS + Lorecivivint</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100 nM</td>
<td>30 nM</td>
</tr>
<tr>
<td><strong>pNF-κB (p105)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total NF-κB</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(p105/p50)</td>
<td></td>
<td></td>
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<tr>
<td><strong>pStat3 (S727)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>pStat3 (Y705)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total Stat3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>β-actin</strong></td>
<td></td>
<td></td>
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</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

Structural Damage

- Wnt gene expression
  - Altered protein levels
  - Chondrocyte differentiation/function

Symptoms

- Inflammatory gene expression
  - Cytokines

Wnt/Mechanical stress/Metabolic/Trauma

- CLK2
  - Alt. splicing

- NF-κB
  - Alt. splicing

- SM04690

- SIRT1

- FOXO1

- DYRK1A

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

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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 planned to start in Q2 2019
Thank you