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"
Lorecivivint (LOR; SM04690) preclinical development

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

- hMSC assays
- Protease assays
- Cartilage Protection
- Cytokine assays
- Anti-inflammatory
- Animal models

**Chondrocyte Regeneration**

**Cartilage Protection**

**Anti-inflammation**

**Sustained Local PK**

**Protease gene expression**

- MMP1
- MMP3
- MMP13
- ADAMTS5

**Cytokine gene expression**

- IL-1β
- TNF-α
- IL-6

**Expected therapeutic level (~30 nM)**

**Improve Joint Health (Animal models)**
Lorecivivint inhibits the Wnt pathway through a unique MOA

- **Yttrium90-Labeled Anti-Fzd10 Antibody**
- **Anti-Fzd7 Antibody**
- **Inhibitor of TCF-CBP Interaction**
- **Soluble Fzd Decoy Receptor**
- **Porcupine Inhibitors**

**Modulation of gene expression**
- Affects Wnt Pathway Proteins (Structural effects)
- Affects Other Pathways (Inflammation)
Lorecivivint is a potent and selective kinase inhibitor

318 kinases tested *in vitro*

<table>
<thead>
<tr>
<th>Kinase Tested</th>
<th>% Inhibition</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

Pre-mRNA

mRNA processing e.g. splicing

mRNA

Translation

Protein

Alternative Splicing

Intron retention

Exon 1
Exon 2
Exon 3
Exon 4
Exon 5

DNA

Pre-mRNA

mRNA

Translation

Protein A

Protein B

Protein C
Cdc-like kinases (CLKs)

Directly affects transcription or alternative splicing of genes

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

Lorecivivint inhibited CLK-mediated SRSF phosphorylation

**Lorecivivint**
*(In vitro CLK2 biochemical kinase assay)*

Log Conc. - Lorecivivint (nM)

% Inhibition

- CLK2
- DMSO

**CLK2 IC$_{50}$ = 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 \textit{in vitro}

Gene expression

- **TCF7**
  - DMSO / LOR

- **ERBB2**
  - DMSO / LOR

- **DVL2**
  - DMSO / LOR

- **AXIN1**
  - DMSO / LOR

Intron retention

\[\rho_{1,2} = -0.76\]

\[\rho_{1,2} = -0.63\]

\[\rho_{1,2} = -0.83\]

\[\rho_{1,2} = -0.66\]

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>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>
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<td></td>
</tr>
<tr>
<td>pSirt1 (Ser47)</td>
<td></td>
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<td></td>
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<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>
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</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>pFoxO1</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total FoxO1</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>β-actin</td>
<td></td>
<td></td>
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</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 measured in hMSCs by Nanostring panel and qPCR

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

**In vitro** siRNA knockdown effects measured in hMSCs 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>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100 nM</td>
<td>30 nM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 nM</td>
<td>3 nM</td>
</tr>
<tr>
<td>pNF-κB (p105)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total NF-κB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(p105/p50)</td>
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<td></td>
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</tr>
<tr>
<td>pStat3 (S727)</td>
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</tr>
<tr>
<td>pStat3 (Y705)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Total Stat3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-actin</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

- Wnt/Mechanical stress/Metabolic/Trauma

**Structural Damage**
- Wnt gene expression
- Altered protein levels
- hMSCs
- Chondrocytes
- Chondrocyte differentiation/function

**Symptoms**
- Inflammatory gene expression
- Synovial fibroblasts
- Cytokines

**Lorecivivint**
- CLK2
- NF-κB
- SIRT1
- FOXO1
- DYRK1A

- Alt. splicing
- Phosphorylation (STAT3, SIRT1)

**Pathways**
- Wnt/Mechanical stress/Metabolic/Trauma
- Inflammatory gene expression

**Significance**
- 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

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Lorecivivint summary

• The intra-nuclear 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