Lorecivivint (SM04690), a Potential Disease-Modifying Osteoarthritis Drug, Inhibits 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

• Lorecivivint is an investigational agent not approved by the FDA or any other regulatory agency
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
- Cytokine assays
- Animal models

Chondrocyte Regeneration

Safranin O  Alcian blue  Type II collagen

Cartilage Protection

Protease gene expression

- MMP1
- MMP3
- MMP13
- ADAMTS5

Cytokine gene expression

- IL-1β
- TNF-α
- IL-6

Anti-inflammation

- Expected therapeutic level (~30 nM)

Sustained Local PK

Improved Joint Health (Animal models)
LOR inhibits the Wnt pathway through a unique MOA

- Yttrium90-Labeled Anti-Fzd10 Antibody
- Anti-Fzd7 Antibody
- Inhibitor of TCF-CBP Interaction
- DYRK1A
- CLKs
- Porcupine Inhibitors
- Soluble Fzd Decoy Receptor
- Wnt5A Mimetic

Modulation of gene expression

Affects Wnt pathway proteins and other inflammatory/structural pathways
LOR is a potent and selective kinase inhibitor

### 318 kinases tested *in vitro*

<table>
<thead>
<tr>
<th>Kinase Tested</th>
<th>% Inhibition LOR (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>
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<tbody>
<tr>
<td>CLK2</td>
<td>98</td>
<td>5.8</td>
<td>1.0</td>
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<tr>
<td>CLK3</td>
<td>100</td>
<td>44.3</td>
<td>7.6</td>
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<tr>
<td><strong>DYRK1A</strong></td>
<td><strong>99</strong></td>
<td><strong>26.9</strong></td>
<td><strong>4.6</strong></td>
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<tr>
<td>DYRK1B</td>
<td>94</td>
<td>41.2</td>
<td>7.1</td>
</tr>
<tr>
<td>GSK3β</td>
<td>92</td>
<td>37.8</td>
<td>6.5</td>
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<tr>
<td>HIPK1</td>
<td>95</td>
<td>33.2</td>
<td>5.7</td>
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<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

Protein

Transcription

Alternative Splicing

Intron Retention

Protein A

Protein B

No Translation
CDC-like kinases (CLKs)

Directly affects transcription or alternative splicing of genes

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

LOR inhibited CLK-mediated SRSF phosphorylation

**LOR**
*In vitro CLK2 biochemical kinase assay*

**SRSF**
*hMSCs in vitro*

**Graphs:**
- **LOR conc. (nM)** vs. % Inhibition for CLK2 and DMSO.
  - CLK2 IC$_{50}$ = 7.8 nM
- **MW** of Ladder, DMSO, 100 nM LOR, 30 nM LOR, 10 nM LOR, 3 nM LOR with bands for pSRSF4, pSRSF5, pSRSF6 and β-actin.
LOR induced intron retention and modulated alternative splicing

RNA sequencing in hMSCs
LOR 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)

**In vitro** DYRK1A biochemical kinase assay

4. Matsuzaki T, Sci Transl Med. 2018

TF: Transcription factor
HD: Histone deacetylase

\(\text{IC}_{50} = 26.9 \text{ nM}\)
LOR inhibited SIRT1 and FOXO1 phosphorylation
Reduced FOXO1 phosphorylation led to increased nuclear FOXO1 levels
CLK2 and DYRK1A knockdowns inhibited the Wnt pathway

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

*P<0.05, **P<0.01, ***P<0.001 vs. siCtrl

*In vitro* siRNA knockdown effects in hMSCs identified by NanoString panel and validated by qPCR
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
LOR decreased phosphorylation of NF-κB and STAT3

**NF-κB and STAT3**

*In vitro* LPS-stimulated synovial fibroblasts

<table>
<thead>
<tr>
<th>Unstimulated</th>
<th>LPS + DMSO</th>
<th>LPS + LOR</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>100 nM</td>
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<tr>
<td></td>
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<tr>
<td></td>
<td></td>
<td>10 nM</td>
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<td>3 nM</td>
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- pNF-κB (p105)
- Total NF-κB (p105/p50)
- pSTAT3 (S727)
- pSTAT3 (Y705)
- Total STAT3
- β-actin
Inhibition of CLK2 and DYRK1A reduced inflammation

**In vitro** siRNA knockdown effects in BEAS-2B cells
Cytokines measured by qPCR
Mean ± SEM; *P<0.05, **P<0.01, ***P<0.001 vs. vehicle
LOR MOA

Osteoarthritis
Increased Wnt signaling/Mechanical stress/Metabolic/Trauma

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

Symptomatic Effects
- Inflammatory gene expression
  - Cytokines (Pain)

Lorecivivint

- Alternated splicing
- SIRT1
- FOXO1
- NF-κB
- CLK2
- DYRK1A

FOXO1: Forkhead box O1
SIRT1: Sirtuin 1
NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells

Stat3: Signal transducer and activator of transcription 3

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
LOR summary

• The intranuclear kinases CLK2 and DYRK1A, dual targets of LOR, are novel targets for modulation of Wnt signaling, chondrocyte biology, and inflammation

• LOR protected cartilage, induced chondrogenesis, and reduced inflammation in vitro and in vivo

• Phase 3 human clinical trials are ongoing
Thank you