Wnt Pathway Modulation via CLK2 and DYRK1A Inhibition by Lorecivivint (SM04690), a Potential Disease-Modifying Treatment for Knee Osteoarthritis

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All authors are employees or shareholders of Samumed, LLC
Redefining osteoarthritis (OA)

- OA is not simply ‘wear and tear’
- Involves abnormal remodeling driven by biomechanical forces and inflammatory mediators
- The joint as an organ is affected, not just cartilage:
  - Articular cartilage degradation
  - Subchondral bone thickening
  - Osteophyte formation
  - Synovial inflammation
  - Ligament and meniscal degeneration
  - Joint capsule hypertrophy
  - Less commonly, changes in periarticular muscles, nerves, bursa, and fat pad
Knee OA: A disease of the whole joint

Disease Targets/Processes
- Bone remodeling
- Bone sclerosis
- Synovitis
- Joint capsule damage
- Cartilage degradation
- Chondrocyte hypertrophy
- Meniscal degradation

Wnt signaling

Healthy Knee

Knee OA

Articular cartilage

Synovial membrane

Bone marrow

Progenitor stem cells
Upregulated Wnt signaling contributes to OA progression

With increased Wnt signaling

- Pro-inflammatory cytokines and catabolic enzymes that drive cartilage degradation and OA symptoms are increased\(^1,2\)
- Progenitor cells in the synovium and subchondral bone form osteocytes rather than chondrocytes\(^3-6\)

3. Loughlin J. Proc Natl Acad Sci USA. 2004
5. Loughlin J. Curr Opin Rheumatol. 2005

Figure adaptations: www.york.ac.uk and Bush J & Beier F. Nature Med. 2013
Lorecivivint inhibits the Wnt pathway through a unique MOA

- **Lorecivivint**
  - Modulation of gene expression
  - Affects Wnt pathway proteins and other pathways (inflammation/structural)

- **Porcupine Inhibitors**

- **Yttrium90-Labeled Anti-Fzd10 Antibody**

- **Soluble Fzd Decoy Receptor**

- **Anti-Fzd7 Antibody**

- **Inhibitor of TCF-CBP Interaction**

- **Wnt5A Mimetic**

**Key Terms:**
- **CLK:** CDC-like kinase
- **DYRK1A:** Dual-specificity tyrosine phosphorylation-regulated kinase 1A

**Pathways:**
- **Wnt**
  - Cytoplasm
  - Nucleus
Lorecivivint inhibits the Wnt pathway through a unique MOA

Modulation of gene expression

Affects Wnt pathway proteins and other pathways (inflammation/structural)

CLK: CDC-like kinase, DYRK1A: Dual-specificity tyrosine phosphorylation-regulated kinase 1A
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

Safranin O  Alcian blue  Type II collagen

Control (DMSO)  SM04690 (30 nM)

Cartilage Protection

Protease gene expression

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

Improved Joint Health (Animal models)

Protease gene expression

Cytokine gene expression

Expected therapeutic level (~30 nM)
Actual scores (mean ± standard errors)
Comparisons of LOR vs. PBO using a baseline-adjusted ANCOVA. Data offset for visual clarity.

WOMAC Pain

mJSW
DYRK1A inhibition reduced inflammation and improved chondrocyte function in *in vitro* OA models.

CLK2 inhibition decreased Wnt pathway activity, induced chondrogenic cell production, and reduced inflammation in *in vitro* OA models.

**Lorecivivint** → **CLK2** → **NFkB** → **Inflammation**

- **Wnt pathway genes:**
  - AXIN2
  - TCF7
  - LEF1
  - TCF4

- **Increased chondrogenic genes:**
  - ACAN
  - CD44
  - COL2A1
  - DOTL1
  - COMP

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

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

In vitro siRNA knockdown effects measured in hMSCs by qPCR
CLK2 and DYRK1A knockdowns 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
• Intranuclear kinases CLK2 and DYRK1A, are novel targets for lorecivivint, causing modulation of Wnt signaling

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

• Phase 3 human clinical trials are ongoing
Thank you
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
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\textsubscript{50} (nM)</th>
<th>Fold IC\textsubscript{50} &gt;CLK2</th>
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<tbody>
<tr>
<td>CLK2</td>
<td>98</td>
<td>5.8</td>
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<td>CLK3</td>
<td>100</td>
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<td>D YorkshireA (DYRK1A)</td>
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<tr>
<td>DYRK1B</td>
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<td>GSK3β</td>
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<tr>
<td>HIPK2</td>
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<td>16.8</td>
<td>2.9</td>
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DNA ➔ Transcription ➔ DYRK1A

Pre-mRNA ➔ mRNA processing e.g., splicing ➔ CLOCK

mRNA ➔ Translation ➔ Protein
Alternative splicing regulation of gene expression

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

DNA → Pre-mRNA → mRNA → Translation → Protein

Alternative Splicing

Intron retention

Protein A

Protein B

No translation