Lubricin
and its Potential as an OA Therapy

Disclosure:
Carl R. Flannery, Ph.D.
is a Sanofi employee & holds stock/options in Sanofi and Pfizer
Biolubricants
The high viscosity of synovial fluid and boundary lubricants on the articular surface maintain healthy cartilage as a near frictionless biomaterial. One of the most important boundary lubricants is a protein by the name of lubricin, encoded by the Proteoglycan 4 (PRG4) gene.
Cartilage is subjected to high load & shear forces

Highly efficient lubrication is imperative for healthy joint function


Normal

Injury/disease progression

Compromised Lubrication

OA
Cartilage lubrication: historical perspective

“… Nature has conveniently covered the rubbing surfaces of the bones with a highly polished layer of cartilage… and has provided a viscous liquid (synovial fluid) to moisten these surfaces.”

“It might seem unduly contentious… to suggest that the synovial fluid of a joint is not a lubricant; this is, however, the object of this paper.”

Lubricin functions as a boundary lubricant and anti-adhesive at joint tissue surfaces, to help prevent wear and degeneration.
Lubricin

- **1970-80s**: Isolated from synovial fluid and characterized as a cartilage lubricating glycoprotein (Swann & Radin, et al.).
- **Early 1990s**: Further characterized by Jay, et al. Gene cloned at Genetics Institute; described as ‘Megakaryocyte Stimulating Factor (MSF)’ precursor.
- **Late 1990s**: Identity of MSF precursor with lubricin/superficial zone protein (SZP) established. Gene name assigned: Proteoglycan 4 (PRG4).

SMB = somatomedin-B (vitronectin)-like
HEP = heparin-binding
HPX = hemopexin-like

\[\Delta = \text{N-linked oligo attachment site}\]
\[\sharp = \text{Lubricating O-linked oligosaccharide: } \beta-(1-3)-\text{Gal-GalNAc} (+/- \text{ sialic acid})\]

\*Jones, et al. JOR 2007
Altered lubricin metabolism and relation to joint pathology

Human lubricin gene mutations are associated with absence of lubricin synthesis (CACP syndrome). Patients exhibit noninflammatory synovial hyperplasia/hypercellularity and subintimal capsular fibrosis, and early onset cartilage degeneration.

Lubricin null mice recapitulate human CACP syndrome phenotype, and exhibit tendon abnormalities as well as elevated joint friction levels.

Perturbations in lubricin expression/function observed in models of joint injury/OA, and in patients with joint injury/OA.

Implications: lubricin supplementation could be beneficial in treating joint disease
## Efficacy of intraarticular (IA) lubricin supplementation in preclinical joint disease/OA models

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<tr>
<th>OA Model</th>
<th>Lubricin/dose</th>
<th>Dosing protocol</th>
<th>Results</th>
<th>References</th>
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<tr>
<td>Rat Meniscal Tear (MT)</td>
<td>rhLubricin (LUB:1); 20µg/joint</td>
<td>1X/wk or 3X/wk for 4 wks starting 1 wk post-Sx</td>
<td>Significantly reduced joint histopathology scores</td>
<td>Flannery, et al. A&amp;R 2009</td>
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<tr>
<td>Rat ACLT</td>
<td>Human synoviocyte or SF lubricin; rhLubricin; 10µg/joint</td>
<td>2X/wk starting 1 wk post-Sx; 32 day study duration</td>
<td>Significantly reduced joint histopathology scores</td>
<td>Jay, et al. A&amp;R 2010</td>
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<tr>
<td>Rat ACLT</td>
<td>Human synoviocyte lubricin; 80µg/joint</td>
<td>Single dose 1 wk post-Sx; 70 day study duration</td>
<td>Significantly reduced uCTX-II levels; gait normalization</td>
<td>Jay, et al. A&amp;R 2012</td>
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<tr>
<td>Rat ACLT + forced exercise</td>
<td>Human SF lubricin; 40µg/joint</td>
<td>Single dose 1 wk post-Sx; 5 wk study duration</td>
<td>Significantly reduced uCTX-II levels; inhibition of chondrocyte apoptosis</td>
<td>Elsaid, et al. OAC 2012</td>
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<tr>
<td>Rat ACLT</td>
<td>rhLubricin; 8µg/joint (+/- IL1ra)</td>
<td>Single dose 1 wk post-Sx; 5 wk study duration</td>
<td>Significant inhibition of chondrocyte apoptosis</td>
<td>Elsaid, et al. OAC 2015</td>
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<td>Rat OVX</td>
<td>rhLubricin; 10µg/joint</td>
<td>2X/week for 4 wks starting day of Sx or 2 wks post-Sx</td>
<td>Significantly reduced joint histopathology and uCTXII levels, sig. inhibition of MMP13 &amp; COLX levels &amp; vascularization in cartilage and TRAP &amp; OSX levels in bone, and normalized bone CT parameters</td>
<td>Cui, et al. Bone 2015</td>
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Recent study also demonstrates chondroprotective effects of helper-dependent adenoviral vector (HDV)-mediated lubricin expression after IA injection in a mouse CLT OA model. Therapeutic model = HDV delivered 2 wks post-Sx, significantly reduced histopathology scores + cartilage volume & surface area at 6 wks post-treatment (Ruan, et al. Sci Transl Med 2013).
Assessing LUB:1 functionality ex vivo

**LUB:1 Binds to Cartilage Surfaces**
Immunostaining of LUB:1

**LUB:1 Lubricates Cartilage**
Custom cartilage friction testing apparatus
*Bonassar Lab, Cornell University*

**LUB:1 is Anti-Adhesive for Synoviocytes**
Therapeutic efficacy of LUB:1 in vivo

Our first proof-of-principle study demonstrated that IA treatment with LUB:1 is chondroprotective in rat meniscal tear OA model

Dosing with 20µg/joint of LUB:1 for 4 weeks starting 1 week post-surgery

1X/week Dosing

<table>
<thead>
<tr>
<th></th>
<th>PBS</th>
<th>LUB:1</th>
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</thead>
<tbody>
<tr>
<td>Score</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>%</td>
<td>100%</td>
<td>82%</td>
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3X/week Dosing

<table>
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<tr>
<th></th>
<th>PBS</th>
<th>LUB:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>%</td>
<td>100%</td>
<td>83%</td>
</tr>
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</table>

Histology: 3X/week Treatment

PBS-treated
Cart. Degen. Score = 4.00
(Group mean = 3.93 ± 0.29)

LUB:1-treated
Cart. Degen. Score = 3.00
(Group mean = 3.08 ± 0.20)

*P<0.05 vs PBS
Focus on cartilage surface – ex vivo injury model

50% compression; 100%/sec → 2d culture post-injury

Effect of injurious compression on cartilage level 1 frictional properties

*P<0.05 vs. control
#P<0.05 vs. non-extracted
Assessing cartilage surface damage in rat meniscal tear (MT) model and effect of LUB:1 treatment

Collaboration with Bonassar Lab (Galley, et al. ORS 2011)

3mm osteochondral cores harvested from Medial Tibial Plateau for testing
Cartilage surface damage in rat MT model (profilometry)
Mechanism(s) of action: beyond lubrication (?)

A prominent feature of lubricin deficiency is synovial hyperplasia/hypercellularity.


• Could lubricin inhibit macrophage infiltration, and thereby influence levels and activities of proinflammatory/pain modulators in the joint?
Assessment of LUB:1 effect on pain (weight-bearing) in rat MT model

- Perform unilateral hind-limb surgery (sham or meniscal tear).
- IA treatment with PBS (vehicle) or LUB:1.
- Measure hind limb weight-bearing distribution.

Collaborative studies with:
Lilly Mark, Garth Whiteside, Wyeth Neuroscience
LUB:1 treatment significantly improves weight bearing in the rat MT model

Diff in weight distribution (g)  
non-Sx versus Sx limb

SHAM + PBS  
MT + PBS  
MT + LUB:1

#p < 0.05 vs. SHAM  
*p < 0.05 vs. PBS

N = 14 animals/group  
3 weeks post-surgery

Collaborative studies with:  
Lilly Mark, Garth Whiteside, Wyeth Neuroscience
Further considerations for the development of lubricin treatment as an OA therapy

- Clinical trials for testing disease-modifying OA drugs (DMOADs) are time consuming → expensive. And to date, unsuccessful...

- Clinical trials for symptom-modifying drugs offer good potential. Relatively rapid; validated and accepted endpoints for pain/function (i.e. WOMAC OA index).

- Local IA dosing advantages include limited systemic exposure and patient compliance. However, turnover/clearance rate must be considered.

- Frequency of administration is a critical factor – regulatory agencies, health authorities/payers advocate minimum 3 mo duration of action.

  - PK studies conducted in rats using [¹²⁵Iodine]-LUB:1 demonstrate tri-phasic disposition profile after single (20µg) IA dose: $T_{1/2} = 4.5h, 1.5d$ and $2.1\text{ wks}$ (Vugmeyster, et al. AAPS J 2012).

    - 6% (1.2µg) of LUB:1 remaining at 48h.
    - At 28d, ~0.05% (0.01µg) of LUB:1 remaining, with localization to joint tissues/cartilage surface.
    - $M_{eff}$ prediction of 5.6µg/knee suggests need to explore extended release formulations.
Acknowledgments

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MIT
Al Grodzinsky & Team

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