Experimental Tendinopathy Treatment with SM04755, a Topical, Small Molecule Inhibitor of the Wnt Pathway

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Wnt signaling and tendinopathy

- Overexpression of Wnt proteins has been demonstrated in human studies and animal models of the disease\textsuperscript{1,2}
- Increased Wnt signaling has been shown to direct tendon derived stem cells away from a tendon cell fate\textsuperscript{1}
- Activated Wnt signaling may lead to increased production of proteases which contribute to tendon degeneration and fibrosis\textsuperscript{2}

Proposed therapy: SM04755

• SM04755 is a topical small molecule that exhibited the following properties in preclinical studies:
  – Sustained tendon and low systemic exposure
  – Tendon regeneration
  – Anti-inflammatory
  – Anti-fibrotic
SM04755 demonstrated sustained local and minimal systemic exposure over 24 hours

- Single topical application of SM04755
- Target concentration achieved and retained in the rat tendon for up to 24 hours with minimal systemic exposure
Regenerative: SM04755 caused tendon-like differentiation of hMSCs

• Human mesenchymal stem cells (hMSCs) treated with BMP+FGF (positive control), vehicle, or SM04755 for 7 days, then stained for markers of tendon differentiation
• SM04755 showed increased tenogenesis compared with vehicle from *in vitro* assay

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**Tenascin C**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Tenascin C Positive Cells %</th>
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<tbody>
<tr>
<td>DMSO 330nM</td>
<td>5 ± 1</td>
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<tr>
<td>DMSO 166nM</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>DMSO 83nM</td>
<td>15 ± 2</td>
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<tr>
<td>BMP+FGF</td>
<td>20 ± 3</td>
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<tr>
<td>SM04755 330nM</td>
<td>25 ± 4</td>
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<tr>
<td>SM04755 166nM</td>
<td>30 ± 5</td>
</tr>
<tr>
<td>SM04755 83nM</td>
<td>35 ± 6</td>
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**SCX A**

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<thead>
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<td>5 ± 1</td>
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<td>DMSO 83nM</td>
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<td>BMP+FGF</td>
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<td>SM04755 330nM</td>
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</tr>
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<td>25 ± 5</td>
</tr>
<tr>
<td>SM04755 83nM</td>
<td>30 ± 6</td>
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**Tenomodulin**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Tenomodulin Positive Cells %</th>
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<tbody>
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<tr>
<td>SM04755 330nM</td>
<td>23 ± 5</td>
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<tr>
<td>SM04755 166nM</td>
<td>28 ± 6</td>
</tr>
<tr>
<td>SM04755 83nM</td>
<td>33 ± 7</td>
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</tbody>
</table>

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EC$_{50}$ = ~200nM

BMP= bone morphogenic protein; FGF=fetal growth factor; quantification of the number of tenocytes: n=9 replicates, mean ± SEM; *p<0.05, **p<0.01, ***p<0.001
Anti-fibrotic: SM04755 inhibited markers of fibrosis

- Human dermal fibroblasts (HDFα) cells treated with TGF-β1 (10 ng/ml) and SM04755 (1 µM) for 48 hrs
- TGF-β1 (positive control) induced the fibrotic markers ACTA-2, PAI-1, CTGF, Col2a1, measured by qRT-PCR.
- SM04755 significantly inhibited these markers compared with control

ACTA-2

PAI-1

CTGF

Col2a1

ACTA-2: gene for alpha smooth muscle actin; PAI-1: plasminogen activator inhibitor 1; CTGF: connective tissue growth factor; Col2a1: Collagen type II, alpha 1; mean ± SEM, n=3 replicates; **p<0.01, ***p<0.001
Anti-inflammatory: SM04755 inhibited LPS-stimulated inflammation

- THP1 human monocytes stimulated with LPS for 24 hrs
- SM04755 dose-dependently inhibited TNF-α and IL-6 secretion

EC50 = ~600 nM
In vivo rat collagenase model of tendinopathy

- A single sham or collagenase injection administered into rat Achilles’ tendon on Day 0, with tendon degeneration evident within hours
- Daily treatment with vehicle or topical SM04755 (10 mg/ml, equivalent to 0.3 mg/cm² SM04755 or 30 mg/ml, equivalent to 0.9 mg/cm² SM04755) from Day 1 through study completion
- Assays for inflammation, tendon regeneration, collagen, and histological scoring were conducted
Anti-inflammation: SM04755 reduced inflammation in an acute *in vivo* rat collagenase model

- SM04755 (0.3 mg/cm²) decreased levels of circulating CXCL1 in peripheral blood following collagenase injection, then SM04755 or vehicle treatment, as measured by ELISA
- SM04755 reduced expression of inflammatory genes in tendon as measured by qRT-PCR. Fold change relative to sham control shown
Regeneration: SM04755 increased tendon regeneration markers in an *in vivo* rat collagenase model

- SM04755 (0.3 mg/cm²) upregulated several tendon regeneration markers on Day 21

**Mean ± SEM, n= 12 rats/group, *p<0.05, **p<0.01, ***p<0.001, ns=not significant**
SM04755 reduced inflammation and improved structure compared with vehicle - *In vivo* rat collagenase model (single injection)

- Collagenase injection followed by daily SM04755 (0.3 mg/cm²), then H&E staining at 21 days
- In comparison with vehicle treatment, SM04755:
  - Significantly reduced inflammatory cells & hemorrhage
  - Demonstrated structural improvement in linearity and density of tendon fibers

![Image of histological sections](image)

**Tendon score**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Total Tendon Score</th>
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<tr>
<td>Sham</td>
<td>n.s.</td>
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<tr>
<td>Vehicle</td>
<td>*</td>
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<tr>
<td>SM04755 (0.3 mg/cm²)</td>
<td>**</td>
</tr>
</tbody>
</table>

Collagenase injection 200 µg

n=4 for sham, n=6 for treatment, mean ± SEM; ***p<0.001, ns=not significant, one-way ANOVA
SM04755 improved pain and weight bearing in an *in vivo* rat collagenase model

- ‘Tendinopathy’ induction in one limb leads to pain and preference for weight bearing on non-affected limb
- Weight distribution measured using incapacitance meter
- SM04755 significantly improved weight bearing on affected limb by Day 7 in comparison with vehicle
- Pain in rats was measured using Von Frey apparatus
- SM04755 significantly decreased pain in treated rats by Day 18, compared with vehicle treatment
Tendon health scores after SM04755 treatment in the acute collagenase tendinopathy model

- 0.9 mg/cm² SM04755 improved tendon health scores over vehicle at Day 14 and Day 21, whereas 0.3 mg/cm² SM04755 showed improvement compared with vehicle only at Day 21.
- Higher dose of SM04755 led to faster recovery in the rat model.
In vivo rat collagenase delayed treatment model of tendinopathy

- Sham or collagenase injections administered into rat Achilles’ tendon on Day -28 and Day -14
- Daily treatment with vehicle or topical SM04755 (10 mg/ml, equivalent to 0.3 mg/cm² SM04755) from Day 0 through study completion
- Assays for inflammation, tendon regeneration, collagen, and histological scoring were conducted
Tendon health scores after delayed SM04755 treatment in the repeat injury delayed treatment collagenase tendinopathy model

In comparison with vehicle treatment, SM04755 (0.3 mg/cm²):

- Reduced inflammatory cells & hemorrhage
- Demonstrated structural improvement in linearity and density of tendon fibers
- Significantly improved tendon health scores

<table>
<thead>
<tr>
<th></th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
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<tbody>
<tr>
<td>Sham</td>
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<td><img src="image9.png" alt="Image" /></td>
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**Histological score**

- Day 7: NS
- Day 14: NS
- Day 21: *P < 0.05*
Summary

• The Wnt pathway modulates the degenerative and fibrotic processes of tendinopathy
• In preclinical tendinopathy models, SM04755 demonstrated:
  – Sustained tendon exposure, with minimal systemic exposure
  – Reduced inflammation
  – Differentiation of progenitor cells into tenocytes
  – Inhibition of fibrotic markers (*in vitro* only)
  – Increased tendon regeneration markers and type 1 collagen
  – Improved tendon structure micro- and macroscopically
  – Improved weight bearing function and pain
• Acute treatment models showed improved tendon health compared with vehicle and indicated a dose-dependent speed of response.
• SM04755 promoted tendon healing compared with vehicle in acute and repeat injury / delayed treatment models.
Thank you