Discovery of a Small Molecule Inhibitor of the Wnt Pathway (SM04755) as a Potential Topical Treatment for Tendinopathy

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Disclosures

- Vishal Deshmukh, Ph.D.
  - Financial disclosure: Samumed, LLC; salary and equity
- Timothy Seo, M.S.
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- Maureen Ibanez, M.S.
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- Luis Dellamary, Ph.D.
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- Josh Stewart, Ph.D.
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- Yusuf Yazici, M.D.
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The Wnt (wingless & int1) pathway is highly conserved across all animals.

- Involved in development of multiple tissues
- Plays a critical role in self renewal and fate determination of mesenchymal stem cells

**Wnt pathway plays a key role in tissue repair and regeneration.**


Wnt in tendinopathy

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

Adapted from Miller et al. Sci. Rep. 6, 27149

1. Liu et al. 2013 Rheumatology
2. Jelinsky et al. 2011 BMC Musculoskeletal Dis
Proposed therapy: SM04755

- SM04755 is a novel, topical, small molecule Wnt pathway inhibitor that exhibited the following properties in preclinical studies:
  - Sustained tendon exposure
  - Low systemic exposure
  - Anti-inflammatory
  - Regenerative
  - Anti-fibrotic
SM04755 demonstrated sustained local and minimal systemic exposure

- Single topical application of SM04755
- Target concentration achieved and retained in the rat tendon for up to 24hrs with minimal systemic exposure.

![Graph showing SM04755 Concentration over time in tendon and plasma](image)
Anti-inflammatory: SM04755 inhibited LPS-stimulated inflammation

- THP1 human monocytes stimulated with LPS for 24hrs
- SM04755 inhibited TNFα and IL6 secretion

**Graphs:**
- IL-6 (pg/mL) vs. Log Conc (μM)
- TNFα (pg/mL) vs. Log Conc (μM)

**EC50 (nM):**
- 527.8
- 609.82
- EC50 = ~600nM
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 from *in vitro* assay

Quantification of the number of tenocytes
n=9 replicates, Mean ± SEM,
*p<0.05, **p<0.01, ***p<0.001

EC$_{50}$ = ~200nM
Fibrosis reversal: SM04755 decreased smooth muscle actin in dermal fibroblasts

- HDFα cells treated with TGFβ1 (10ng/ml) for 48hrs to induce fibrosis, followed by treatment with SM04755 for 48hrs
- Cells fixed and stained for αSMA (fibrotic marker)
- SM04755 reversed αSMA expression

Mean ± SEM, n=3

EC₅₀ = ~125 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 (10mg/ml, equivalent to 0.3 mg/cm²) from Day 1 till study completion
Anti-inflammation: SM04755 reduced inflammation in an acute *in vivo* rat collagenase model

- SM04755 (10mg/ml) 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.

Mean ± SEM, n=6 for sham, n=4 for vehicle & SM04755
NS=not significant; * p<0.05, **p<0.01
Regeneration: SM04755 increased tendon regeneration markers in an *in vivo* rat collagenase model

- SM04755 (10mg/ml) upregulated several tendon regeneration markers on Day 21

![Graphs showing expression levels of Tenomodulin, TenascinC, and Scleraxis](image)

*Mean ± SEM, n= 12 rats/group, *p<0.05, **p<0.01, ***p<0.001, ns=not significant*
Regeneration: SM04755 improved Type I to III collagen ratios in an *in vivo* rat collagenase model

- Normal tendons are mainly type I collagen\(^1\)
- Type III collagen is thinner and upregulated in tendinopathy.
- Ratio of type I:type III is low in tendinopathy\(^2\)
- SM04755 (10 mg/ml) improved the ratio of type I:type III collagen by Day 21

\[ n=12/\text{group (10 mg/ml). } ^*p<0.05, \quad ^{**}p<0.001 \]

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1. Lui YF, et.al 2010 *Rheumatology*
2. Hanson AN, et.al. 1983 *Anal Biochem*.
Regeneration: SM04755 treatment increased type I collagen and improved tendon structure

- Collagenase injection followed by daily SM04755 (10 mg/ml), then histology at 21 days
- Type I (normal)- Yellow/Orange | Type III (thinner, disorganized collagen during injury)- Green
- SM04755 treatment increased type I collagen and improved tendon structure compared to vehicle.

Representative images
SM04755 reduced inflammation and improved structure in *in vivo* rat collagenase model

- Significant reduction in inflammatory cells & hemorrhage
- Structural improvement in linearity, shape and density of tendon fibers

Mean ± SEM, n=4 for sham, n=6 for vehicle & SM04755

***p<0.001, ns=not significant
SM04755 improved weight bearing after collagenase injection in *in vivo* rat collagenase model

- ‘Tendinopathy’ induction in one limb leads to 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 to vehicle

![Graph showing weight bearing comparison](image)

Mean ± SD, n= 10 rats/group, 6X per measure
*p<0.05, **p<0.01, ***p<0.01 vs. vehicle
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 in multiple species
  – Reduced inflammation
  – Differentiated progenitor cells into tenocytes
  – Inhibited fibrotic markers (*in vitro only*)
  – Increased tendon regeneration markers and type 1 collagen
  – Improved tendon structure micro- and macroscopically
  – Improved weight bearing function
Current Status and Next Steps

• Phase 1, single blind, topical study in healthy subjects for SM04755 program began Q4 2016
  – Primary objectives: safety and tolerability, dose ranging
  – Secondary objective: plasma pharmacokinetics
  – Abraded and non-abraded skin

• Phase 2, double blind, topical study in sub-acute lateral epicondylitis in development
Thank you samumem