Discovery of a Small Molecule Inhibitor of the Wnt Pathway (SM04690) as a Potential Disease Modifying Treatment for Knee Osteoarthritis

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### Background
- Knee osteoarthritis (OA) is characterized by destruction of articular cartilage, subchondral bone alterations, and synovitis.
- At a cellular level, Wnt signaling affects OA pathogenesis in joints by influencing inflammation, cartilage breakdown, and bone/cartilage formation. Increased Wnt signaling induces stem cells to differentiate into osteoblasts, and decreased signaling induces chondrogenesis.\(^2\)
- Samumed is developing a small molecule Wnt pathway inhibitor, SM04690, as a potential disease modifying OA drug (DMOAD) injected into the knee.
- Preclinical studies of SM04690 were conducted to evaluate chondrogenesis, anti-inflammation, cartilage protection, and joint health.

### Methods
- Wnt pathway inhibition was measured by qPCR in human mesenchymal stem cells (hMSCs).
- Chondrogenesis was evaluated using hMSCs by qRT-PCR and immunocytochemistry.
- Cytokine induced protease release and glycosaminoglycan (GAG) breakdown in chondrocytes was measured by qRT-PCR and dimethylthymine blue (DMMB) assay.
- Anti-inflammatory activity was evaluated by measuring TNF-α and IL-6 secretion using ELISA in synovial fibroblasts stimulated with IL-1β. Pre-inflammatory cytokines (TNF-α, IL-1α, IL-1β, IL-2, IL-6, IL-8, IL-17A, IL-17F, IFN-γ, & PGE2) were evaluated by ELISA in T and B cell co-cultures stimulated with superantigen or LPS, compared to vehicle or two benchmark immunosuppressants (cyclosporin A and prednisolone).
- Pharmacokinetics of SM04690 in plasma and joint were evaluated following intra-articular (IA) injection in rats.
- In vivo activity of SM04690 was evaluated in a rat model: anterior cruciate ligament transaction with medial meniscal tear (ACLT+pMMx) using Osteoarthritis Research Society International (OARSI) scoring and biomarker measurement in knee and plasma by qPCR and ELISA.

### Results
- **SM04690 regenerates cartilage in the ACLT+pMMx model of rat OA**
  - Figure 3. ACLT + pMMx induced OA in treated rats (single IA injection of vehicle or SM04690). (a) Representative images of rat knee stained with Safranin O-Fast/Green 12 weeks post treatment. (b) Joint score (OARSI scoring system). (n=7) OA, Vehicle; (n=6) OA, SM04690 (3μg). (c) Gene expression of chondrocyte markers in rat cartilage 4 weeks post treatment, measured by qRT-PCR. (d) Total sulphated GAG levels relative to tissue weight, measured by DMMB assay. (n=7 for vehicle and n=8 for treatment, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001)

- **SM04690 protected cartilage in the ACLT+pMMx model of rat OA**
  - Figure 4. ACLT + pMMx induced OA in treated rats (single IA injection of vehicle or SM04690). (a) Protease gene expression in rat cartilage 4 weeks post treatment (qRT-PCR). (n=7) Vehicle; (n=8) OA, Treatment, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001. (b, c) Circulating COMP and PIAPP measured by ELISA. (n=12 rats, Mean ± SEM, *p<0.05)

- **SM04690 inhibited inflammatory responses in co-culture systems in vitro with comparable or greater potency than Cyclosporin A and Prednisolone**
  - Figure 5. (a) IL-6 and TNF-α inhibition in human synovial fibroblasts stimulated with IL-1β and treated with SM04690 for 24hrs measured by ELISA. (b) Inflammatory cytokine inhibition in human synovial fibroblasts stimulated with IL-1β and treated with SM04690 for 24hrs (qRT-PCR), (n=3, Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001). (c) Comparison of in vitro anti-inflammatory activity of SM04690 with cyclosporin A and prednisolone performed on DiscoverX BioMAP® platform (scale 0.5-0, weak and 5-highly potent activity). SM04690 demonstrated comparable or greater potency than the two standard-of-care drugs across several anti-inflammatory assays.

### Conclusions
- SM04690, a small molecule Wnt pathway inhibitor, induces chondrogenesis, protected chondrocytes from catabolic breakdown, increased cartilage thickness and improved joint health in a rat model of knee OA.
- Additionally, potent anti-inflammatory effects of SM04690 observed in various cell types may provide beneficial effects in the treatment of OA.
- Human clinical trials with SM04690 are ongoing.

### References

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