Discovery of a Small Molecule Inhibitor of the Wnt Pathway (SM04690) as a Potential Treatment for Degenerative Disc Disease

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Background

- Degenerative Disc Disease (DDD), a major cause of low back pain, is characterized by degeneration of intervertebral discs (IVDs)[1], which are composed of a central nucleus pulposus (NP) surrounded by collagenous annulus fibrosus (AF) and cartilaginous endplates. IVDs are essential for load-bearing, mobility, flexibility, anchoring, and shock absorption of the vertebra.[2,3]
- The NP is comprised of progenitor cells that can differentiate into chondrocyte-like cells to form a proteoglycan and collagen-rich extracellular matrix (ECM), responsible for hydration and IVD function[4-8]; loss of NP cellularity and hydration results in decreased disc height and function.[1-2, 9-10]
- Wnt signaling plays a key role in IVD development and maturation. Excessive Wnt signaling results in inhibition of NP cell proliferation, upregulation of ECM degrading enzymes, and apoptosis of NP cells, which leads to IVD degeneration and DDD.[1, 11-13]
- Treatment of DDD is limited to analgesics or surgery aimed at relieving symptoms. No current therapy can reverse disc degeneration.[2,14]
- Samumed is developing SM04690, a potent small molecule Wnt signaling inhibitor, as a potential injectable therapeutic for the treatment of DDD.

Methods

- To identify Wnt signaling inhibitors, a small molecule chemical library was screened in a cellular Wnt pathway-based β-catenin/TCF-responsive reporter assay in SW480 colon cancer cells.
- *In vitro* proliferation of NP cells isolated from rat coccygeal discs, treated with vehicle or SM04690 for 5 days, was measured by cell doubling index (CDI=cell number/initial cell number/days).
- Differentiation of NP progenitor cells into chondrocyte-like NP cells with 12 days of vehicle or SM04690 treatment was measured by Alcian blue staining and absorbance based quantification.
- Pharmacokinetics were evaluated by intradiscal injection in rats and rabbits, followed by LC-MS analysis of compound concentrations in the disc and plasma.
- Rat coccygeal IVD needle puncture was used as a DDD model.
- Injured discs were radiographed pre-surgery and 1 week (dosing point), 4 weeks, and 6 weeks post-surgery.
- Safranin O/Fast Green or Masson's Trichrome stained discs were histologically evaluated by blinded observers using a disc scoring system[15] based on grading of the integrity of AF, border between AF and NP, and cellularity and matrix of NP. Disc height index (DHI) was calculated by averaging the anterior, middle, and posterior portions of the disc height and dividing by the average height of the adjacent vertebral body.

Results

SM04690 demonstrated specific and potent inhibition of Wnt signaling

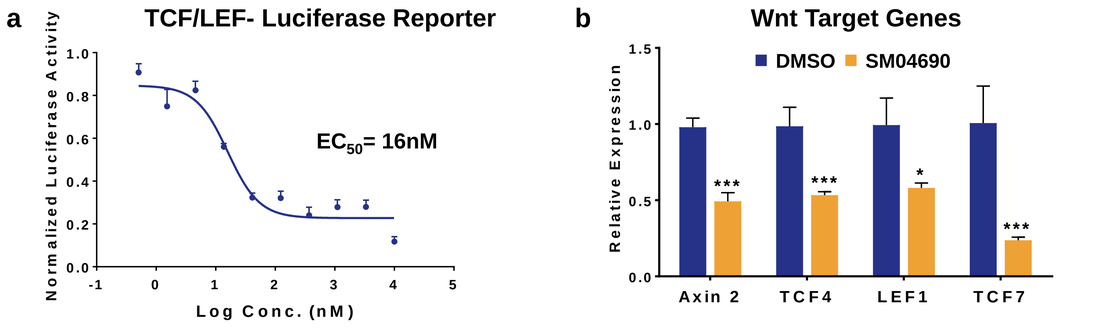

Figure 1. (**a**) Dose response of SM04690 treatment of SW480 cells transduced with TCF/LEF promoter-driven luciferase reporter. (**b**) Expression of genes in the Wnt pathway in hMSCs following treatment with SM04690 or DMSO for 24hrs as measured by qRT-PCR. (n=3, Mean ± SEM, * p<0.05, ***p<0.001).

SM04690 stimulated NP-derived progenitor cell proliferation

- CDI for primary NP-derived progenitor cells was ~2-fold higher in cells treated with SM04690 compared to DMSO.
- Increased Alcian blue staining indicated the presence of chondrocyte-like cells after 12 days of treatment with SM04690.

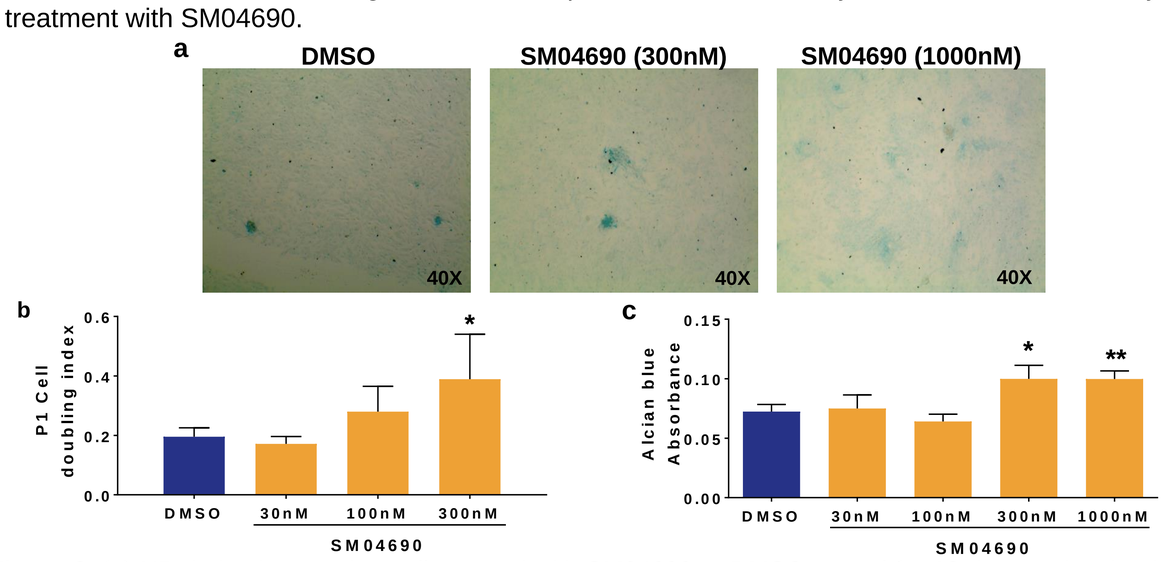

Figure 2. (**a**) NP-derived progenitor cells treated with SM04690 or DMSO control for 12 days and stained with Alcian blue. (**b**) CDI for NP-derived progenitor cells treated with various doses of SM04690 or DMSO control for 5 days. (**c**) Quantification of Alcian blue staining for the cells in (**a**). (n=3, Mean ± SD, *p<0.05, **p<0.01, one-way ANOVA).

Results

SM04690 demonstrated sustained residence time in intervertebral discs and minimal systemic exposure

- Rats injected with 10 μL of SM04690 (3.3 μg/mL, 33 μg/mL, or 330 μg/mL).
- Residence time of >60 days observed after a single intradiscal injection of SM04690 (33 μg/mL or 330 μg/mL).
- 33 μg/mL, corresponding to 0.33 μg drug/disc, demonstrated sustained release and was cleared at 180 days.

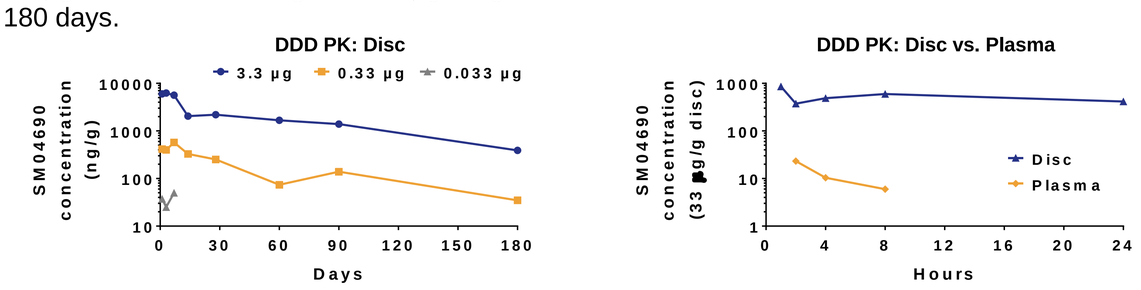

Figure 3. Pharmacokinetics of SM04690 in the rat disc following a single intradiscal injection of SM04690 at various doses.

A single intradiscal injection of SM04690 improved disc health and shape in a rat *in vivo* model of degenerative disc disease

- Needle puncture injury; single injection of SM04690, 33 μg/mL, received one week after injury.
- SM04690-treated discs showed less fragmented AFs, larger NP and ECM areas, and more NP cells as compared to vehicle-treated discs.
- Histology scores measuring AF, AF and NP border, cellularity of the NP, and content of matrix of the NPs were significantly lower for SM04690-treated discs as compared to vehicle.

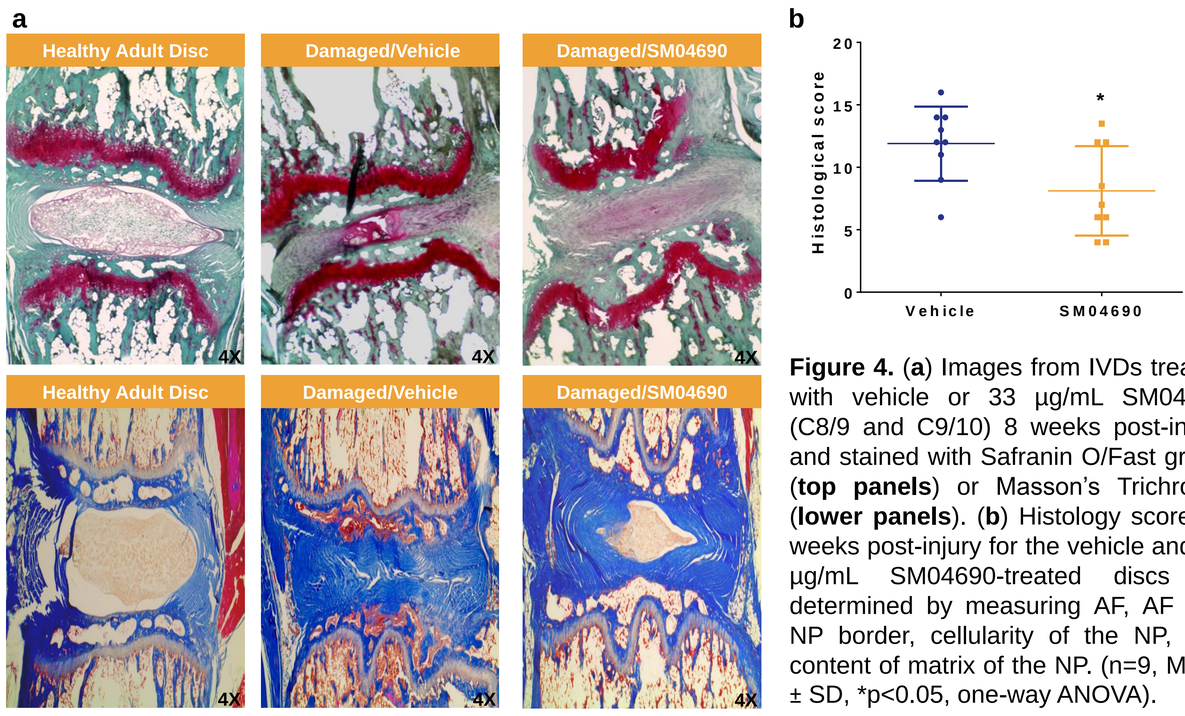

Figure 4. (**a**) Images from IVDs treated with vehicle or 33 μg/mL SM04690 (C8/9 and C9/10) 8 weeks post-injury and stained with Safranin O/Fast green (**top panels**) or Masson's Trichrome (**lower panels**). (**b**) Histology scores 8 weeks post-injury for the vehicle and 33 μg/mL SM04690-treated discs as determined by measuring AF, AF and NP border, cellularity of the NP, and content of matrix of the NP. (n=9, Mean ± SD, *p<0.05, one-way ANOVA).

A single intradiscal injection of SM04690 maintained disc height in a rat *in vivo* model of degenerative disc disease

- Significantly higher percent DHI observed in the SM04690-treated group compared to vehicle, 5 weeks post-treatment.

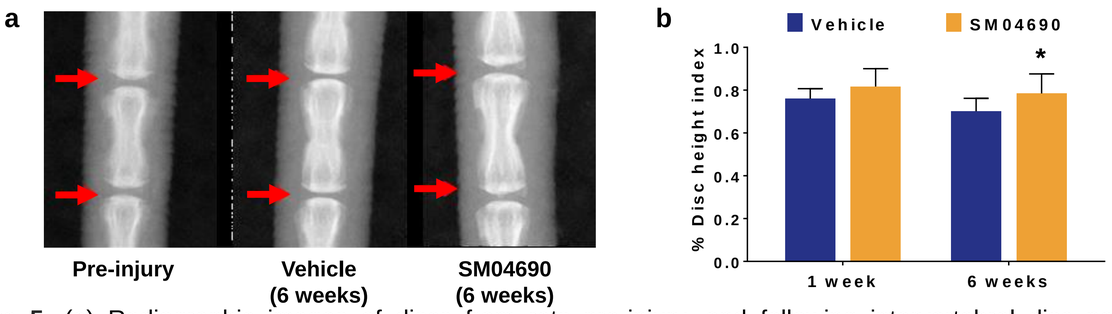

Figure 5. (**a**) Radiographic images of discs from rats pre-injury, and following intervertebral disc needle puncture and treatment with either vehicle or 33 μg/mL SM04690 (red arrows indicate C8/9 and C9/10 discs). (**b**) Percent DHI calculated based on radiographic images at 1 and 6 weeks post-injury and compared to pre-operative (pre-injury) radiographic images. (n=9, Mean ± SD, *p<0.05, student's t-test).

Conclusion

- SM04690 induced the proliferation and differentiation of NP-derived progenitor cells *in vitro* and *in vivo* (Figure 2 and 4).
- Single intradiscal injection of SM04690 had sustained residence time in the disc and minimal systemic exposure in rats (Figure 3).
- Single intradiscal injection of SM04690 improved disc height, health, and shape after injury *in vivo* in a rat model of DDD (Figure 4 and 5) compared to vehicle controls.
- SM04690 regenerated the NP areas and IVDs in this *in vivo* model of DDD.
- An Investigational New Drug application for SM04690 in DDD is open and human trials are planned for 2017.

References

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