Preclinical Evidence for SM04690, a Small Molecule Wnt Pathway Inhibitor, as a Potential Treatment for Degenerative Disc Disease

Vishal Deshmukh, Charlene Barroga, and Yusuf Yazici
Samumed, LLC, San Diego, CA

Background

- Degenerative disc disease (DDD), a major cause of low back pain, is characterized by degeneration of intervertebral discs (IVDs), which are composed of central nucleus pulposus (NP) surrounded by collagenous annulus fibrosus (AF) and cartilaginous endplates.[1]
- The NP contains 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; loss of NP cellularity and hydration results in decreased disc height and function.[1,2]
- Wnt signaling plays a key role in IVD development and growth.[3] Excessive Wnt signaling results in inhibition of NP cell proliferation, upregulation of pro-inflammatory cytokines and ECM degrading enzymes, and apoptosis of NP cells, which lead to IVD degeneration and DDD.[4-6]
- Pharmacological treatment of DDD is limited to analgesics and/or surgery aimed at relieving symptoms and experimental biologic treatments with as yet unknown efficacy.[7] No current FDA approved therapy has been shown to reverse disc degeneration.[1]
- SM04690, a potent small molecule Wnt pathway inhibitor, is being developed 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.
- Anti-inflammatory activity was evaluated by measuring a panel of secreted pro-inflammatory cytokines from peripheral blood mononuclear cells (PBMCs) stimulated with super-antigen (sAg) or IgM.
- Effects on fibrosis were assessed in TGF-β-stimulated human dermal fibroblasts (HDFα) by measuring smooth muscle actin (αSMA).
- *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 following 12 days of vehicle or SM04690 for 5 days was measured by Alcian blue staining and absorbance based quantification.
- Pharmacokinetics was 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 (immediately prior to dosing), 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[8] 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

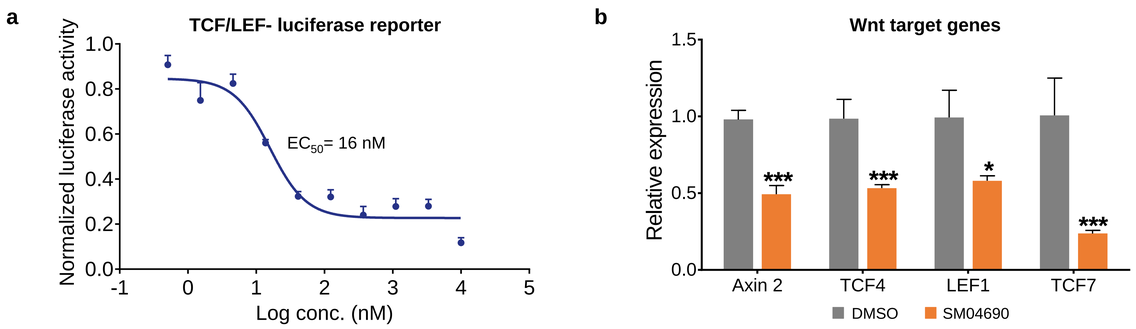

Figure 1. (**a**) Dose response of SM04690 treatment of SW480 cells transduced with TCF/LEF promoter-driven luciferase reporter. (**b**) Expression of genes in the Wnt pathway in hMSCs following treatment with SM04690 (30 nM) or DMSO for 24 hrs as measured by qRT-PCR (n=3, mean ± SEM, * p<0.05, ***p<0.001 compared with vehicle [DMSO]).

SM04690 inhibited T and B cell inflammatory responses in co-culture systems

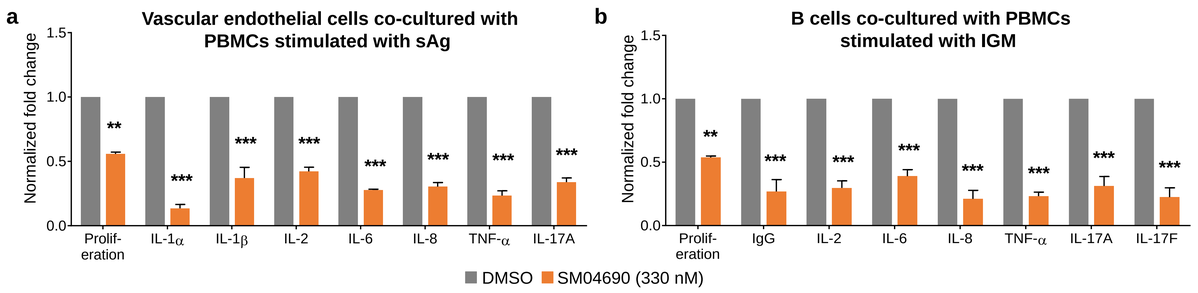

Figure 2. Inhibition of pro-inflammatory cytokine secretion by SM04690 in (**a**) vascular endothelial cells co-cultured with human PBMCs, stimulated with sAg and (**b**) B cells co-cultured with human PBMCs and stimulated with IgM, as measured using the DiscoverX BioMAP® platform (n=3, mean ± SEM, **p<0.01, ***p<0.001 compared with vehicle [DMSO]).

SM04690 inhibited fibrosis in human dermal fibroblasts *in vitro*

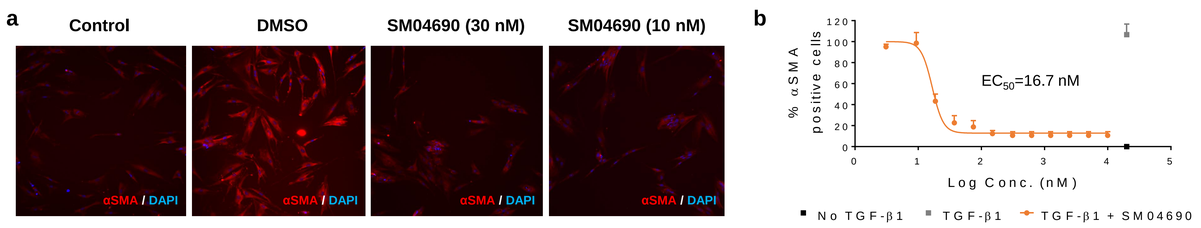

Figure 3. (**a**) HDFα cells treated with TGF-β1 (10 ng/mL) for 48 hrs to induce fibrosis, followed by treatment with various doses of SM04690 for 48 hrs. (**b**) Quantification of the number of cells positive for αSMA in (**a**).

Results

SM04690 stimulated NP-derived progenitor cell proliferation and differentiation

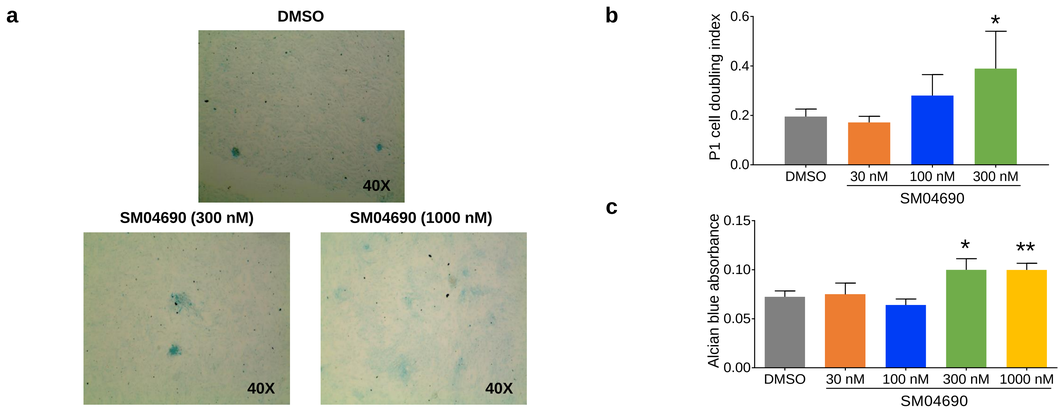

Figure 4. (**a**) NP-derived progenitor cells treated with various doses of SM04690 or DMSO control for 12 days and stained with Alcian blue. (**b**) Cell doubling index (CDI) for NP-derived progenitor cells treated with various doses of SM04690 or DMSO control for 5 days. (**c**) Quantification of Alcian blue staining for the cells in (**a**). n=3, mean ± SD, *p<0.05, **p<0.01, one-way ANOVA compared with vehicle [DMSO].

SM04690 demonstrated sustained residence time in IVDs and minimal systemic exposure

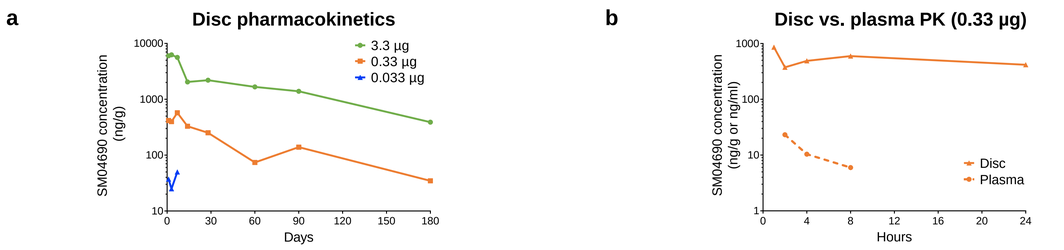

Figure 5. (**a**) Pharmacokinetics of SM04690 in the rat disc following a single intradiscal injection of SM04690 at various doses. (**b**) Pharmacokinetics of SM04690 in disc compared with plasma for the 0.33 µg dose. SM04690 showed sustained exposure in the disc and rapid plasma clearance.

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

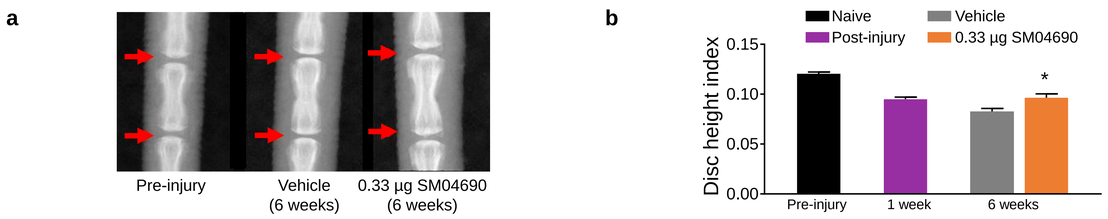

Figure 6. (**a**) Radiographic images of discs from rats pre-injury and following intervertebral disc needle puncture and treatment with either vehicle or 0.33 µg SM04690 (red arrows indicate C8/9 and C9/10 discs). (**b**) DHI based on radiographic images at 1 (pre-treatment) and 6 weeks post-injury (5 weeks post treatment; n=9, mean ± SD, *p<0.05 compared with vehicle).

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

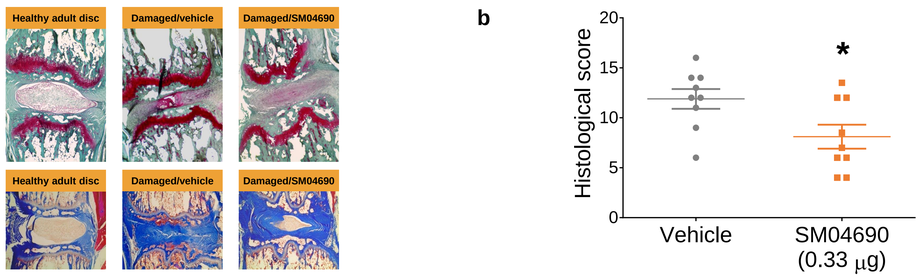

Figure 7. (**a**) Images from IVDs treated with vehicle or 33 µg/mL SM04690 (C8/9 and C9/10) 8 weeks post-injury and stained with Safranin-O/Fast green (top panels) or Masson's Trichrome (lower panels). All images 40x magnification. (**b**) Histology scores 8 weeks post-injury for the vehicle and 33 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.

Conclusions

- SM04690, a small molecule inhibitor of the Wnt signaling pathway, demonstrated potent anti-inflammatory and anti-fibrotic activity *in vitro.*
- SM04690 induced the proliferation and differentiation of NP-derived progenitor cells *in vitro.*
- A single intradiscal injection of SM04690 had sustained residence time in the disc and minimal systemic exposure in rats.
- In a rat model of DDD, a single intradiscal injection of SM04690 improved disc height, health, and shape after injury *in vivo* compared with vehicle controls.
- SM04690 improved the NP and IVD structure in this *in vivo* model of DDD compared with vehicle control.
- SM04690 has potential as a regenerative treatment for DDD.
- A phase 1 trial is ongoing in subjects with DDD (clinicaltrials.gov identifier NCT03246399).

References

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9381 Judicial Drive ● San Diego, CA 92121 ● Email: info@samumed.com