

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

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**ABSTRACT INTRODUCTION:** Degenerative Disc Disease (DDD), is a major cause of low back pain. It is characterized by degenerative changes in the intervertebral disc, nucleus pulposus (NP) and cartilage matrix, resulting in decreased disc height, and loss of shock-absorption capability. Treatment of DDD is limited to pain management with NSAIDs and analgesics or surgical procedures including disc replacement. Treatments are aimed at relieving symptoms - no current therapy can reverse disc degeneration. Wnt signaling in disc progenitor cells potentially plays an important role in DDD by possibly regulating degenerative changes, and likely repair mechanisms such as the proliferation and differentiation of resident NP cells. SM04690, a novel, small-molecule, Wnt pathway inhibitor was evaluated in a series of preclinical studies to determine its potential to induce proliferation and differentiation of primary NP cells, thereby promoting disc healing.

**METHODS:** Wnt pathway inhibition was measured using a cell based luciferase reporter assay controlled by a Wnt-responsive promoter. *In vitro* proliferation of NP cells from rat coccygeal discs, treated with vehicle or 30nM-1000nM of SM04690 for 5 days was measured by cell doubling index (CDI= cell number/initial cell number/days). Differentiation of NP cells into “chondrocyte-like” NP cells with vehicle or SM04690 treatment for 12 days was measured by Alcian blue staining and absorbance based quantification. Pharmacokinetics were evaluated by intradiscal injection in rats and rabbits, followed by analysis of compound concentrations in the disc and plasma. *In vivo* efficacy following a single intradiscal injection of SM04690, was evaluated in a rat coccygeal intervertebral disc “needle puncture” model, using radiographic measurement of disk height index (DHI = DH/vertebral height), and, as a measure of ‘disc health’, histological scoring (total 4-16) of sections stained with Safranin O/Fast Green or Masson’s Trichrome for integrity of annulus fibrosus (AF), border between AF and NP, cellularity, and matrix of NP.

**RESULTS SECTION:** SM04690 demonstrated potent ( $EC_{50} \sim 11nM$ ) and selective inhibition of Wnt signaling. SM04690 induced dose-dependent proliferation of NP cells *in vitro* with CDI ~2 fold higher in cells treated with SM04690 as compared to vehicle ( $P<0.05$ ). Cells treated with SM04690 also showed significantly increased Alcian blue absorbance, indicating differentiation to “chondrocyte-like” cells ( $P<0.01$ ) and production of proteoglycan components of the extracellular matrix (ECM). Single intradiscal injection of SM04690 resulted in disc concentrations  $>EC_{50}$  for  $>180$  days, with minimal systemic exposure or toxicity, measured as behavioral, health, gross morphology, and microscopic adverse changes. In the *in vivo* rat DDD model, a single intradiscal injection of SM04690 (0.066 $\mu$ g/disc) increased Safranin O/Fast Green- and Masson’s Trichrome- stained cartilage matrix (Figure A), and decreased histology scores ( $P<0.05$ ; Figure B), indicating reduced AF lamellar disorganization and fragmentation, larger NP area, increased number of NP cells and increased ECM vs. vehicle control. Radiographic measurement of disc height demonstrated significantly increased % DHI ( $P<0.05$ ; Figure C), in SM04690 treated rats as compared to vehicle control, indicating an improvement in the disc health.

**DISCUSSION:** Wnt signaling plays a critical role in the progression of DDD as well as NP cell differentiation and disc regeneration. SM04690, a small molecule Wnt pathway inhibitor promoted proliferation and differentiation of NP cells *in vitro*. In a rat model of DDD, SM04690 partially reversed disc degeneration with regenerated NP cells and cartilage matrix, and improved disc height, health and shape compared to vehicle, with minimal exposure in the plasma or systemic toxicity. These results suggested that SM04690 has potential as a treatment for DDD.

**SIGNIFICANCE:** There are no current therapeutic options for DDD which attempt to reverse the disease or promote long-term disc healing. Since the NP is the most affected region in DDD, harnessing endogenous NP progenitor cells to produce proteoglycans and regenerate discs could help rebuild and maintain the architecture in a degenerated disc. Sustained, localized disc exposure was obtained following single intradiscal injection of SM04690. SM04690 demonstrated efficacy in a pre-clinical rodent model for DDD. These data suggest that SM04690 has potential as a disease modifying therapy for DDD.

IMAGES AND TABLES:

**Figure 1. SM04690 stimulates differentiation of NP cells and improves disc height and health in a rat model of DDD.**

