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

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Introduction: Degenerative Disc Disease (DDD) involves degeneration of intervertebral disc structure, including the nucleus pulposus (NP), annulus fibrosus (AF), and cartilage matrix. Wnt signaling plays an important role in DDD, regulating the proliferation and differentiation of resident NP cells. SM04690, a novel, small-molecule, Wnt pathway inhibitor was evaluated in preclinical studies to determine its potential to induce proliferation and differentiation of NP cells, thereby promoting disc healing.

Methods: Wnt pathway inhibition was measured using a cell-based reporter assay. *In vitro* proliferation of rat NP cells was measured by cell doubling index (CDI= cell number/initial cell number/days). Differentiation of NP cells into 'chondrocyte-like' NP cells was measured by Alcian blue absorbance based quantification. The effect on myofibroblast differentiation was assessed in TGF-β stimulated human dermal fibroblasts by measuring smooth muscle actin (αSMA) using immunocytochemistry. Pharmacokinetics was evaluated by intradiscal injection in rats, 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 of DDD[1] using radiographic measurement of disc height index (DHI = disc height/vertebral height), and histological scoring (total 4-16) of Safranin O- stained sections for AF integrity, AF and NP border, cellularity, and NP matrix.

Results: SM04690 was a potent (EC_{50}=11 nM) inhibitor of Wnt signaling. *In vitro*, SM4690 induced dose-dependent proliferation of NP cells with CDI ~2-fold greater than vehicle (p<0.05). Cells treated with SM04690 also showed significantly increased Alcian blue absorbance vs. vehicle (P<0.01), indicating differentiation to "chondrocyte-like" cells and production of proteoglycan components of the extracellular matrix (ECM). SM04690 inhibited TGF-β1-induced expression of α-SMA (EC_{50}=16.7 nM). Single intradiscal injection of SM04690 resulted in disc concentrations >EC_{50} for >180 days, with minimal systemic exposure or toxicity, measured as behavioral health, morphology and microscopic changes. In the rat DDD model, a single intradiscal injection of SM04690 (0.33µg/disc) increased Safranin O/Fast Green stained cartilage matrix (Figure A), and decreased histology scores at 8 weeks (P<0.05; Figure B), indicating reduced AF lamellar disorganization and fragmentation, larger NP area, increased cellularity of NP and increased ECM vs. vehicle control. Radiographic measurement of disc height demonstrated significantly increased DHI at 6 weeks (P<0.05; Figure C) in SM04690-treated rats as compared to vehicle.

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 and prevented myofibroblast differentiation *in vitro*. In a rat model of DDD, single intradiscal injection of 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. A phase 1 clinical trial is ongoing.

References: Issy AC, Castania V, Castania M, Salmon CE, Nogueira-Barbosa MH, Bel ED, Defino HL. Experimental model of intervertebral disc degeneration by needle puncture in Wistar rats. Braz J Med Biol Res. 2013;46(3):235-244.

Figure: SM04690 stimulated differentiation of NP cells and improved disc height and health in a rat model of DDD