3-Month Safety and Dose-Dependent Efficacy of a Cell/Scaffold Injection Containing Human Disc-Derived Progenitor Cells in a Rabbit Model of Degenerative Disc Disease

Lara I. Silverman, PhD¹, Galina Dulatova, PhD¹, Terry Tandeski, PhD¹, John Ward, PhD¹, Subba Chintalacharuvu, PhD², Tricia Wolff², Jairo Nunes, DVM², Antwain Howard, DVM², Kellie Howard, PhD³, Mark Parrish⁴, Kevin Foley, MD⁴.

¹Discgenics, Inc, Salt Lake City, UT, USA, ²Covance, Inc, Greenfield, IN, USA, ³Covance, Inc, Seattle, WA, USA, ⁴Semmes-Murphey Neurologic Institute, Memphis, TN, USA.

Disclosures:
L.I. Silverman: 3A; Discgenics, Inc.. 4; Discgenics, Inc.
G. Dulatova: 3A; Discgenics, Inc.. 4; Discgenics, Inc.
T. Tandeski: 3A; Discgenics, Inc.
J. Ward: 3A; Discgenics, Inc.
S. Chintalacharuvu: None. T. Wolff: None. J. Nunes: None. A. Howard: None. K. Howard: None. M. Parrish: None. K. Foley: 1; Medtronic. 3B; Medtronic, ArthroCare, Nuvasive, Discgenics., 4; Discgenics, Truevision.

Introduction: Degenerative disc disease (DDD) is a primary cause of back pain, which affects over 65 million people in the US and costs over $100B in care annually. The disease is characterized by a progressive breakdown of extracellular matrix that can result in decreased disc height, including a loss of proteoglycan in the central nucleus pulposus (NP) region that causes the appearance of a ‘dark disc’ via MRI imaging. Treatments for DDD are limited and do not result in repair/regeneration of the disc. We have identified a method to isolate progenitor cells directly from human disc tissue and form a therapeutic cell population known as ‘discogenic cells’. These cells are multipotent, express a unique profile of surface markers, and produce large amounts of endogenous proteoglycan and collagen. A pilot xenogenic study in rabbits demonstrated the safety and efficacy after 6 weeks of a therapy that included these cells with a viscous hyaluronic acid (600-800kDa, 0.9% concentration) carrier, known as injectable discogenic cell therapy (IDCT). The goal of the present xenogenic study was to test long-term (2 months, 3 months) in vivo safety, efficacy and dose sensitivity of this treatment. Disc height changes, MRI imaging and histology were used to monitor injury and repair. qPCR was used to quantify human versus rabbit cells in the discs, and identify species-specific gene expression of matrix molecules. Finally, serology and pathology was performed to assess safety. We hypothesized that disc height index and NP% would increase with treatment in a dose-dependent manner, with sustained improvements over 3 months. Also, no immune response would be noted, and gene expression of native cells would change with injury and be lower than implanted cells. This study will help to identify the optimal dosing to be translated to human clinical trials, and also will elucidate the underlying mechanism of action behind the observed efficacy.

Methods: Using a previously validated model, lumbar discs of 24 New Zealand rabbits were accessed surgically and punctured with a needle to induce degeneration (n=3 discs/animal) (all animal work performed after approval of private IACUC). After two weeks, L3-L4 and L5-L6 were injected with therapy (3,000, 10,000, 30,000 or 100,000 human cells), L4-L5 received either cell-free scaffold control or were left as untreated control, and L2-L3 was maintained as an uninjured control, resulting in n=6/group/timepoint. Every 2 weeks for 12 weeks, disc height was measured by 18 boney landmarks using x-ray images and normalized to week 0 values, resulting in a disc height index percentage (DHI%). MRI images were acquired using a Varian 7T DirectDrive MRI spectrometer: First, T2-weighted sagittal and transverse images were taken to identify the L1-L6 discs, and then transverse images taken for T2-mapping (TR/TE = 3000/20 ms, first TE = 20 ms, number of echoes = 8, NEX = 2, FOV = 90x90 mm2, 256x256 matrix, 5 slices and 3 mm thickness). Using ImageJ, the percentage of NP was identified by setting a threshold of >100 ms to define water-rich NP versus annulus fibrosus, and normalizing the NP area by the whole disc (identified by ImageJ freeform tool) to determine %NP.

After 9 and 12 weeks, the rabbits were euthanized; a disc per condition was harvested, flash frozen and
processed using the Qiagen AllPrep RNA/DNA extraction kit. The DNA was quantified using an internally validated qPCR-based assay to distinguish human Alu repeats and rabbit RNASEH1 in a single sample. Gene expression was quantified using internally validated TaqMan gene expression assays for aggrecan, collagen 2 and the housekeeping gene TBP for both human and rabbit species. Results were normalized to the housekeeping gene and then to the uninjured control. Fresh discogenic cells from the laboratory were also analyzed, for comparison. The remaining 5 discs were prepared for histology in paraffin. Sections were stained with H&E or Alcian blue, and assessed by a board-certified pathologist. Finally, blood serum was collected from three 12-week animals and analyzed for 20 common health indicators, including total protein and albumin/globulin ratio. Statistics: 1-way and 2-way ANOVAs using Tukey’s post-hoc test was used for the DHI% data and T2 map quantification (NP area), with significance set at $p < 0.05$ and standard deviation graphed as error bars.

**Results:** Disc height index decreased by 30-40% 2 weeks post-injury. Over the course of 2 and 3 months, the injured control and scaffold control DHI% did not improve. The IDCT-treated discs improved in disc height to 78% ± 9 after 2 weeks in the 2 month cohort and 77% ± 10% after 4 weeks in the 3 month cohort (Figure 1A) compared to pre-treatment (week 2) ($p < 0.05$), with sustained efficacy over time and no additional changes. Also, all IDCT doses (3,000 to 100,000 cells) were comparable (Figure 1B), with some fluctuations at week 12. T2-weighted transverse images of 9-week discs (Figure 2A) and T2 intensity maps (Figure 2B) showed marked changes with injury. However, the T2-map quantification of NP area did not improve with treatment (Figure 2C). DNA quantification showed that human cells did not persist to 9 weeks. Gene expression of aggrecan in the native cells was slightly up-regulated with the presence of hyaluronic acid (1.53 ± 0.47 compared to 1.1 for injured control), and collagen 2 expression was down-regulated for all conditions explored (0.08 ± 0.06) compared to untreated discs (data not shown). In contrast, discogenic cells increased in aggrecan and collagen 2 expression 251- and 174-fold over the course of the in vitro culture process. Histologically, injury resulted in clear changes in NP architecture such as loss of cellularity and increase in matrix density, which was partially repaired after treatment (Figure 3A). The IDCT-treated nucleus pulposus was rich in proteoglycan (alcian blue staining) (Figure 3B), showed no evidence of immune cells in the disc or surrounding cartilage and bone, and did not contain fibrotic material, bone, fat, articular cartilage, or other abnormal tissue. Rabbit serology was normal for all markers tested, including protein levels (data not shown).

**Discussion:** A strongly powered, 3-month rabbit study was used to demonstrate the safety and efficacy of a novel cell therapy to treat DDD. No dose dependency was noted despite 33-fold difference in cell dose, contrary to our hypothesis. Disc height improved immediately after injection and cells did not persist to 9 weeks, suggesting a short engraftment period in vivo. Further work is on-going to determine the length of engraftment. The formation of new disc material did not correlate to higher intensity T2 signal, suggesting that T2 intensity alone may be not sufficient to determine efficacy in clinical trials. Finally, no safety concerns were noted, supporting further development of this therapy for human clinical trials. A limitation of the study is the use of small animals; preliminary large animal studies findings have been similar, with more powered studies pending. This work presents relevant preclinical evaluation criteria for degenerative disc disease therapies, and provides supporting preclinical data for the development of IDCT for human clinical testing.

**Significance:** This work describes a broad range of safety and efficacy measures useful in evaluating novel therapies (cellular and non-cellular) for DDD and other intervertebral disc conditions. Further, this research supports the pre-clinical development of a novel therapy for the treatment of DDD in humans.

**Acknowledgments:**

**References:**
Figure 2: MRI Imaging (A) T2-weighted images of uninjured (left) and injured (right) discs, with corresponding T2-map below (B). %NP area of disc for various conditions. Star indicates difference from uninjured control (p < 0.05, n=6).

Figure 3: Histology (A) H&E images of uninjured control, injured control and IDCT (5X, EP – endplate, NP – nucleus pulposus). (B) Alcian blue staining of NP.
Figure 1: Disc Height Changes (A) 2 and 3 month DH1%. (B) 3 month DH1% by dose. (C) Representative radiograph. Star indicates difference from 2 week, line indicates difference from end-to-end (p < 0.05, n=24 for combined doses, n=6 other groups).