Comparative Viability, Potency and In vivo Efficacy of a Fresh or Cryopreserved Cell Therapy for the Treatment of Degenerative Disc Disease

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INTRODUCTION

• Cryopreserving cells provides many benefits for clinical use and commercialization, such as long-term storage, off-the-shelf usability, and the ability to complete safety and functional testing of the cells prior to human dosing.

• Our lab has identified a method to isolate progenitor cells directly from human disc tissue and create therapeutic cells known as discogenic cells to treat degenerative disc disease (Figure 1). We have optimized a formulation and method for cryopreserving these cells that is appropriate for clinical use.

• We compared the viability and potency of fresh and cryopreserved discogenic cells both in vitro (using a potency assay described at the 2013 ASGCT meeting) and in vivo (using a Gottingen minipig™ model).

• We hypothesized that the cryopreserved cells would be comparable to the fresh cells, which have been extensively tested previously.

METHODS

• Discogenic cells were combined with Profreeze™ and 7.5% DMSO. Cells were either tested prior to freezing (fresh), or after controlled rate freezing (cryopreservation) and storage at < -150°C.

• Fresh and cryopreserved cells (post-thaw) were stained with live/dead fluorescent dye. Post-thaw, the metabolic activity of cells after 24 hours was assayed using Alamar Blue resazurin dye metabolic assay (n=6).

• Matrix formation (potency) was assessed by incubating a high concentration of cells in DMEM/F12 with 5% FBS, 10 ng/ml TGFβ and 100 mM dextrose (n=6) for 10 days. The samples were digested with papaip and the amount of GAG was quantified via DMMB assay.

• For the in vivo study, using a previously validated model, three lumbar discs of 4 Gottingen minipigs™ were injury (note: all animal work approved by private IACUC). After four weeks, each animal received either no treatment (injured control), gel control (1% w/v of high MW hyaluronic acid), 100,000 fresh cells with gel, or 100,000 frozen cells with gel (n=3/condition). Cryoprotectant was not removed prior to dosing. X-rays were performed every 4 weeks and disc height index calculated using 18 boney landmarks. Also, four weeks after injection, the discs were processed for parafin histology and stained with H&E, Safranin O and Masson’s trichrome.

RESULTS

• The cells remained highly viable after cryopreservation, as seen qualitatively via live/dead staining (Figure 2, A) and quantitatively using Alamar Blue across 3 lots (Figure 2, B).

• The in vitro potency assay for GAG production demonstrated significant and similar accumulation of GAG before and after cryopreservation (Figure 2, C).

• In vivo, the control discs continued to decrease in height after treatment, but both cell-treated discs showed static or improved disc height and were not significantly different from each other (Figure 3, A). Histology showed variable morphology across pig discs, with no unique differences between fresh and cryopreserved treatments (Figure 3, B).

SUMMARY/CONCLUSIONS

• Maintaining viability and potency after cryopreservation is a crucial step towards commercializing a cell therapy product.

• These studies demonstrate the cryopreservation protocols are appropriate and support the advancement of cryopreserved discogenic cells into clinical trials as a treatment for degenerative disc disease.