**ABSTRACT**

Cardiosphere-derived cells (CDCs) are immunomodulatory, limit fibrosis, and promote tissue regeneration. Preclinical studies showed that intramyocardial administration of CDCs improved cardiac function and exercise capacity, while increasing long-term survival in a Duchenne muscular dystrophy model. This motivated the HOPE clinical trial with positive results after intracoronary administration of CDCs. Recently, a similar improvement was also seen in mdx mice after systemic delivery of CDCs. Here, we aim to evaluate multiple systemic administration of allogeneic CDCs (allo-CDCs) in promoting additional improvement in exercise capabilities in mdx mice and to analyze the immune response after multiple administrations of allo-CDCs.

Repeated systemic dosing of allo-CDCs resulted in increased exercise capability after two administrations in mdx mice. CDCs showed a low immunogenic profile with weak production of alloantibodies against the recipient. These data demonstrate that repeat-dosing of CDCs is effective in producing additional exercise improvement in mdx mice. Although allo-CDCs are recognized by the immune system, their low immunogenic profile and immune-regulatory capabilities allow them to be effectively administered multiple times.

**RESULTS**

**Allogeneic CDCs Increase Exercise Capacity of mdx Mice**

We analyzed the therapeutic activity of allo-CDCs delivered intravenously (jugular) into mdx mice. Improvement in exercise capacity was measured after the first and second allo-CDC dose, 6 weeks apart (Figure 5A). Analysis of serum alloantibodies revealed weak production of alloantibodies against the allo-CDCs (Figure 5B).

**Steroid Administration Does Not Impact CDC Efficacy**

Daily steroid administration is standard of care for DMD patients. To measure the efficacy of CDCs in the presence of steroids, we administered 1 mg/kg/day prednisolone to mdx mice during the week of CDC administration. Mice underwent treadmill exercise testing every 3 weeks to limit the learning effect seen with weekly exercise (Figure 6).

**CONCLUSIONS**

- Allogeneic CDCs given intravenously are effective in increasing exercise capacity after first and second administration.
- Steroid administration does not impact the efficacy of allogeneic CDCs.
- CDCs have a low immunogenic profile and a strong immunomodulation of T-cells that can limit the inflammatory process observed in DMD patients.
- These findings support clinical evaluation of systemic administration and repeat-dosing of CAP-1002 in patients with DMD to potentially maximize efficacy.

**REFERENCES**


**BACKGROUND**

Duchenne Muscular Dystrophy (DMD) is Associated with Chronic Inflammation

DMD is an X-linked recessive disorder affecting roughly 1 in 3,500 males. The disease is caused by mutations in the dystrophin gene, causing a truncation of dystrophin. Without dystrophin, the cell membrane is destabilized, eventually leading to cell apoptosis and chronic inflammation.

**Figure 1.** Dystrophin (green) is a large protein that anchors the sarcolemma to the actin cytoskeleton and has an important structural role during muscle contraction and relaxation.

**Cardiosphere-Derived Cells Are an Allogeneic Cell Therapy Prepared from Human Heart Tissue**

CAP-1002 is an allogeneic cell therapy product based on the production of CDCs.

**Figure 2.** CDC manufacturing scheme. A) Exp apt-desen derived cells (EDCs) are produced from donor heart tissue and expanded to produce a master cell bank (MCB). B) Cardiospheres are formed from EDCs. C) CDCs are produced by culturing the cells obtained after seeding the cardiospheres.

**CAP-1002 is Immunomodulatory and Regenerative**

The immunomodulatory and regenerative properties of CDCs (Figure 3) have previously been effective in vitro and in vivo models.

**RESULTS**

**Systemic Therapeutic Effects with IC Delivery in HOPE Trial**

Recently, positive systemic skeletal muscle effects were reported in the HOPE I clinical trial (Figure 4). The positive results observed in HOPE on both cardiac and skeletal muscle function repeated additional studies exploring new delivery routes and regimens that can maximize therapeutic benefit of CDCs, allow for multiple administrations, and reduce patient administration discomfort.

**Figure 6.** CDC efficacy in the presence of steroids. A) A single injection of allo-CDCs was given at 0 weeks. Steroids were administered daily during the week of CDC injection. Treadmill exercise capacity was measured at weeks 0, 3, and 6. B) Immune cells in spleen were measured for each group at the end of the experiment.

**Figure 7.** CDCs limit proliferation of in vivo activated human T lymphocytes.

**Human CDC Immunology in vitro**

CDCs show a low immunogenic phenotype with expression of class I HLA, CD86 co-stimulatory antigen, and the immuno-regulatory marker PD-L1 (Figure 7). CDCs limit proliferation of in vitro activated human T lymphocytes.

**Figure 3.** CDC mechanism of action. CDCs secrete extracellular vesicles containing bioactive molecules that regulate different responses involved in tissue regeneration.