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Systemic Dose-Finding Study with AAV-Mediated γ-Sarcoglycan Gene Therapy for Treatment of Muscle Deficits in LGMD2C Mice

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BACKGROUND

- Limb girdle muscular dystrophy (LGMD) refers to a group of autosomally inherited neuromuscular dystrophies that are genetically diverse.[1] Each subtype represents a unique mutation and a compilation of symptoms.
- Limb girdle muscular dystrophy type 2C (LGMD2C) results from mutations in the γ-sarcoglycan (SGCG) gene, causing loss of functional protein.[1] It presents as progressive muscular dystrophies starting in the girdle muscles before extending to lower and upper extremity muscles, and can also present in the diaphragm and heart, resulting in respiratory and cardiac failure in specific patient subtypes.[2]
- There are currently no approved disease-modifying therapies for LGMD2C.
- Adeno-associated virus (AAV)-mediated gene transfer therapy has shown early signs of potential to treat sarcoglycanopathies. Key considerations include a systematic and stepwise evaluation of safety, transduction, expression, localization, cellular impact, and clinical function.
- With these considerations in mind, the self-complementary rAAVrh74.MHCK7.hSGCG construct (SRP-9005) was designed to restore functional SGCG to muscles:
  - rAAVrh74 vector: Displays robust muscle (skeletal and cardiac) tissue tropism and has relatively low level of pre-existing immunity[3]
  - MHCK7 promoter: Regulates and drives transgene expression selectively in skeletal and cardiac muscle. Includes an alpha-myosin heavy chain enhancer to drive especially strong expression in cardiac muscle[4]
  - hSGCG transgene: Carries full-length SGCG codon-optimized cDNA
- The SGCG-deficient mouse model (SGCG-/-) lacks the SGCG gene and exhibits complete loss of the SGCG protein, and its phenotype recapitulates the clinical pathological features seen in patients with LGMD2C (eg, cardiomyopathy, necrosis, fatty infiltration, central nucleation, fibrosis, atrophy and hypertrophy). Due to deteriorating muscle function, untreated SGCG-/- mice experience increased fatigue, reduced diaphragm force outputs, and decreased overall activity.
- In this study, we analyzed SGCG-/- mouse models treated with 3 different dosages of SRP-9005.

OBJECTIVE

- Test efficacy and safety of systemic gene transfer of SRP-9005 in SGCG-/- mice by IV delivery through the tail vein.
- Establish MED (minimum effective dose) and proposed clinical dose moving to pivotal study.

METHODS

ANIMAL MODELS

- All procedures were conducted in accordance with approval by The Sarepta Gene Therapy Center of Excellence Institutional Animal Care and Use Committee

scAAVrh74.MHCK7.hSGCG CONSTRUCT

- Self-complementary (sc) adeno-associated virus vector, scAAVrh74, containing a codon-optimized full-length human SGCG transgene, driven by a muscle-specific promoter

TREATMENT COHORTS (n=30)

- SGCG-/- mice, with BL6 genetic background, recapitulate the LGMD2C disease phenotype
- Systemic delivery through tail vein
  - Saline injected BL6 WT (n=6)
  - Saline injected SGCG-/- (n=6)
  - Low dose injected SGCG-/-: 4.63e12 vg/kg (8.94e10 vg total dose)† (n=6)
  - Mid dose injected SGCG-/-: 1.85e13 vg/kg (3.63e11 vg total dose)† (n=6)
  - High dose injected SGCG-/-: 7.41e13 vg/kg (1.26e12 vg total dose)† (n=6)
- Mice treated at 4 weeks old, endpoint 12 weeks post-treatment

†Dose calculated based on linear qPCR

ENDPOINT ANALYSES

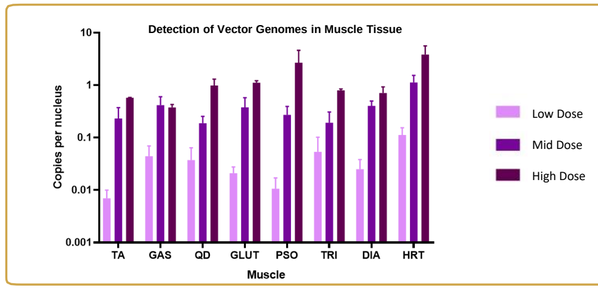
- Biomarker expression (immunofluorescence)
- Transduction (qPCR-vector genomes)
- Histology (central nucleation, fiber diameters)
- Function (physiology)
- Safety (clinical chemistries)

RESULTS

DOSE ESCALATION OF SRP-9005 IN SGCG-/- MOUSE

- A single systemic injection of SRP-9005 resulted in successful systemic delivery as shown by biodistribution of vector genome copies (**Figure 1**).

Figure 1. Expression analysis: Biodistribution of vector genome copies in skeletal muscle



RESULTS (CONT'D)

- IV administration of SRP-9005 to SGCG-/- mice in the presence of significant histopathology in the muscle resulted in transgene expression throughout tibialis anterior (TA) limb, diaphragm (DIA), and heart (HRT) muscles across doses (**Figure 2**).
- SGCG-/- mice show absent or reduced sarcolemma expression of α-sarcoglycan (SGCA), β-sarcoglycan (SGCB), and δ-sarcoglycan (SGCD), components of the dystrophin-associated protein complex (DAPC) (**Figure 3**).
- Treatment with SRP-9005 increased SGCA, SGCB, and SGCD subunit expression at the sarcolemma in SGCG-/- mice, demonstrating a dose-dependent restoration of DAPC proteins (**Figure 3**).

Figure 2. Expression analysis: Immunofluorescence staining on skeletal muscle

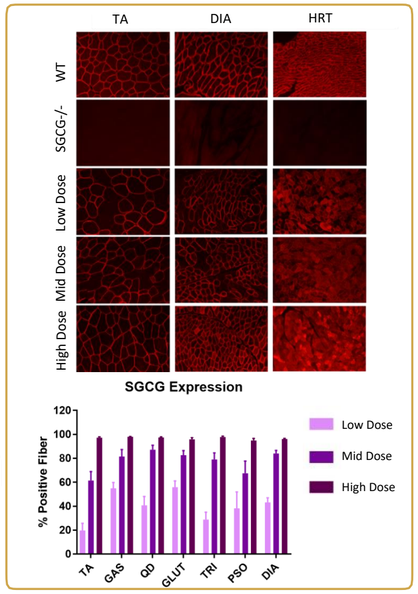
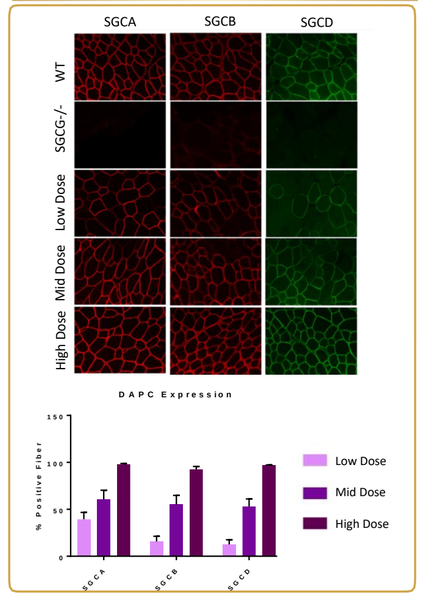


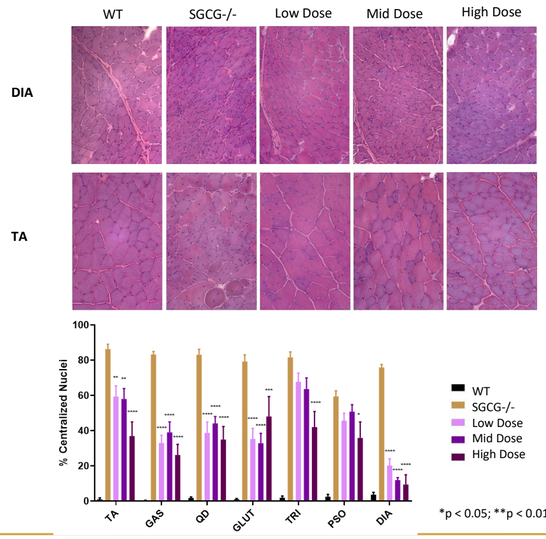
Figure 3. Expression analysis: Restoration of DAPC proteins



- SGCG-/- mice presented significant histopathology in the muscle. After treatment with SRP-9005, overall muscle pathology improved, and decreases in central nuclei were observed (**Figure 4**).

Figure 4. Evidence of severe muscle pathology in muscle and histological benefit following gene transfer

H&E staining for assessment of histopathology of SGCG-/- muscle

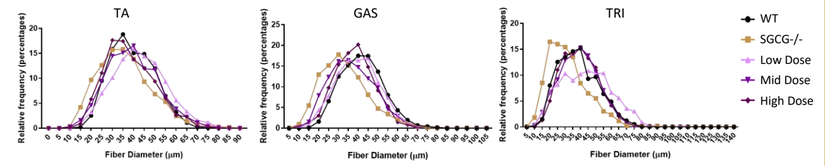


*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001

RESULTS (CONT'D)

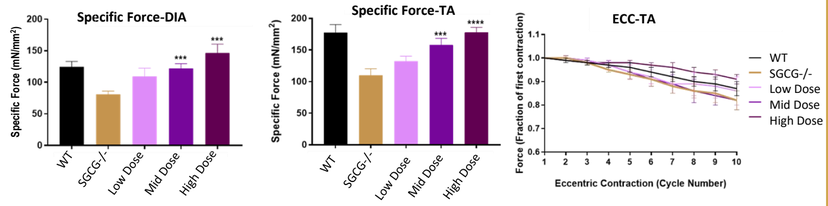
- An increase in fiber diameter in all three dosages was seen, indicating normalized fiber size similar to WT fibers in TA, gastrocnemius (GAS), and triceps (TRI) muscles (**Figure 5**).

Figure 5. Quantitative muscle morphometrics



- Functional improvement was observed with significantly increased muscle strength (force production) and resistance to contraction-induced injury in the TA and DIA muscles (**Figure 6**).

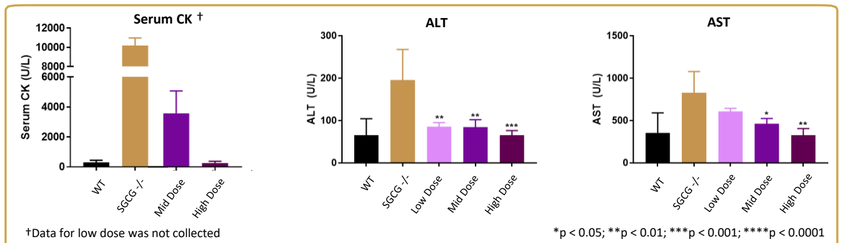
Figure 6. Functional analysis: Protection of force output following treatment of SGCG-/- mice



*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001

- SRP-9005 was associated with a decrease in CK levels. Liver enzymes (ALT and AST) returned within the normal limits for mice after treatment (**Figure 7**).

Figure 7. Safety: CK and clinical chemistry analysis



†Data for low dose was not collected

*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001

CONCLUSIONS

- Collectively, the results of this study demonstrate the ability for AAV gene therapy to be efficacious and provide therapeutic benefit, including widespread high-level protein expression and histopathologic and functional improvements, across multiple doses in a dose-dependent manner.
- A dose-dependent response was also seen in the restoration of sarcoglycan complex proteins (α-, β-, and δ-), indicating the ability to restore additional DAPC proteins.
- The results of this study indicate that the proposed dose of 7.41e13 vg/kg enables the most robust SGCG expression and effective functional restoration, without any adverse effects on non-target tissues, and therefore will inform dose selection in moving towards pivotal study of SRP-9005.

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