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# Treatment of Aged Mice and Long-term Durability of AAV-Mediated Gene Therapy in Two Mouse Models of LGMD

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## BACKGROUND

- The sarcoglycanopathies are a subset of autosomal recessive limb-girdle muscular dystrophies (LGMD) resulting from mutations in the sarcoglycans (α, β, γ, and δ-SG) leading to protein deficiency, loss of formation of the sarcoglycan complex, and loss of stabilization of the dystrophin-associated protein complex (DAPC).
- Sarcoglycanopathies present 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.
- 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 AAV.MHCK7.hSGCB construct was designed to restore functional β-sarcoglycan to muscles:
  - AAVrh74 vector: Displays robust muscle (skeletal and cardiac) tissue tropism and has a relatively low level of pre-existing immunity
  - 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
  - hSGCB transgene: Carries full-length β-sarcoglycan cDNA
- Outstanding questions remain around the ability to treat older, more severely affected muscle; and the long-term durability of the AAV viral vector.
- In this study, we analyzed a mouse model of LGMD2D (α-sarcoglycan) treated at an older age with an AAVrh74.tMCK.hSGCA vector and a model of LGMD2E (β-sarcoglycan) treated with an AAVrh74.MHCK7.hSGCB vector following a long-term endpoint.

## OBJECTIVES

- Provide evidence of AAV gene therapy to be efficacious and provide therapeutic benefit when delivered to older age, more severely diseased muscle with presence of muscle degeneration and fibrosis (>20% in *SGCA*-/- skeletal muscle).
- Demonstrate long-term durability of AAV-mediated gene transfer therapy.

## METHODS

### ANIMAL MODELS

- Mice were maintained under standardized conditions on a 12:12-hour light:dark cycle, with food and water provided ad libitum.

### AGED MOUSE - ALPHA-SARCOGLYCAN (SRP-9004)

#### Treatment cohorts

- *SGCA*-/- mice recapitulate the LGMD2D disease phenotype
- Systemic delivery through tail vein of *SGCA*-/- mice
  - Vector (SRP-9004) *SGCA*-/- (n=5)
  - LRS injected *SGCA*-/- (n=5)
  - LRS injected BL6 WT (n=4)
- Mice treated at 12 months old, endpoint 6 months post-treatment, 18 months of age

#### Endpoint analyses

- Biomarker expression (immunofluorescence, western blot)
- Transduction (qPCR-VGs)
- Histology (central nucleation, diameters, fibrosis)
- Function (activity cage, physiology)
- Safety (clinical chemistries, histopathology review)

### LONG-TERM DURABILITY - BETA-SARCOGLYCAN (SRP-9003)

#### Treatment cohorts

- *SGCB*-/- recapitulate the LGMD2E disease phenotype
- Systemic delivery through tail vein of *SGCB*-/- mice
  - Vector (SRP-9003) *SGCB*-/- (n=5)
- Mice treated at 4 weeks old, endpoint >24 months (27 months) post-treatment

#### Endpoint analyses

- Biomarker expression (immunofluorescence)
- Transduction (qPCR-VGs)
- Histology (central nucleation, diameters)

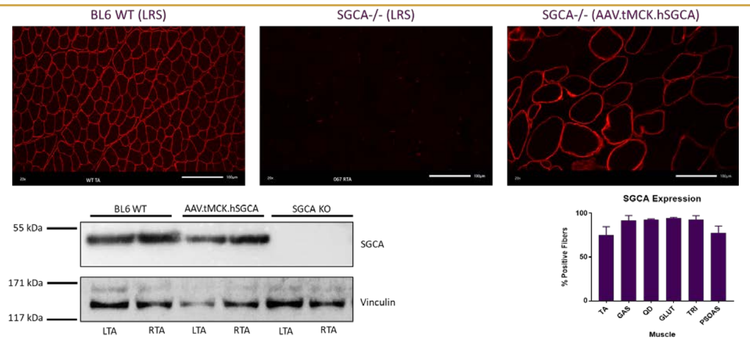
## RESULTS

### AGED MOUSE - ALPHA-SARCOGLYCAN (SRP-9004)

- 12-month-old *SGCA*-/- mice present with severe dystrophic pathology in muscle with high central nucleation (indicating significant degeneration/regeneration cycles) and substantial levels of existing fibrotic tissue (>20%) (**Figure 2a-SGCA KO**).
- IV administration of rAAVrh74.tMCK.hSGCA to 12-month-old *SGCA*-/- mice in the presence of significant histopathology in the muscle resulted in widespread high-level protein expression in muscles throughout the lower limb, upper limb, and proximal torso muscles, including the DIA and heart (**Figure 1**).

## RESULTS

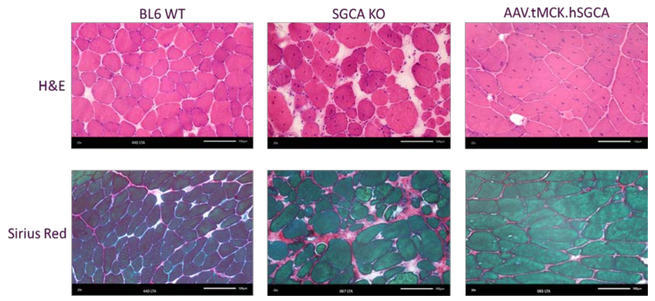
### Figure 1. Expression analysis: Immunofluorescence staining and western blot on skeletal muscle indicating biomarker expression in aged, severely diseased muscle



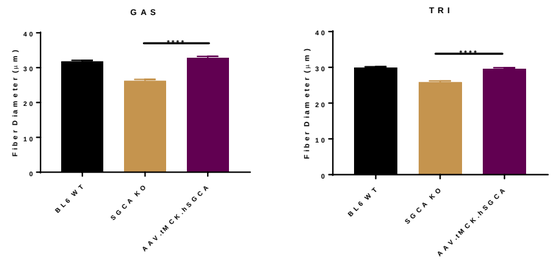

- Overall muscle pathology improved (**Figure 2a**).
- A significant increase in fiber diameter indicating normalized fiber size similar to WT fibers in gastrocnemius (GAS) and triceps (TRI) muscles were seen along with decreases in central nuclei (**Figure 2b**).
- We also noted a reduction in levels of fibrosis compared to untreated controls (**Figure 2c**).

### Figure 2. Evidence of severe muscle pathology in aged muscle and histological benefit following gene transfer

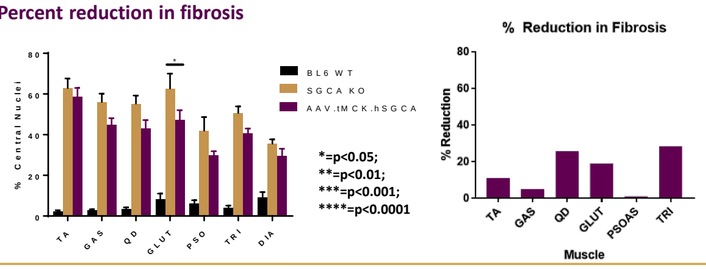
#### 2a. H&E staining, picrosirius red staining on skeletal muscle




#### 2b. Quantitative muscle morphometrics



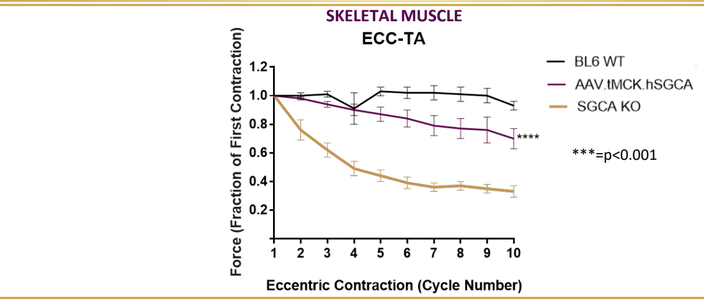

#### 2c. Percent reduction in fibrosis




## RESULTS (CONT'D)

- Functional improvement was observed with significantly increased resistance to contraction-induced injury in the TA muscle (**Figure 3**).

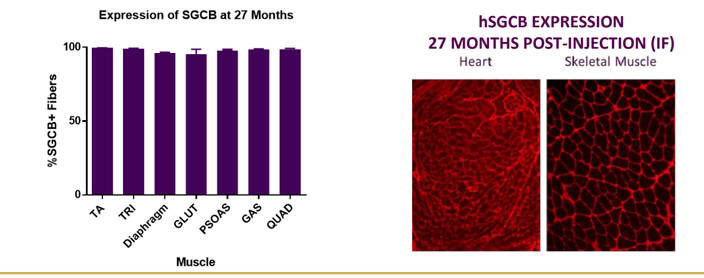
### Figure 3. Functional analysis: Protection of force output following long-term treatment of aged *SGCA*-/- mice with severely diseased muscle



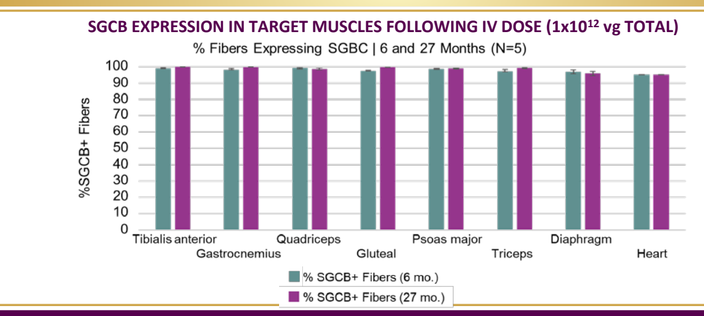

### LONG-TERM DURABILITY - BETA-SARCOGLYCAN (SRP-9003)

- Durability of AAV gene therapy was established in *SGCB*-/- mice treated systemically with rAAVrh74.MHCK7.hSGCB.
- Using qPCR, we detected high-level vector genome copy numbers across all transduced muscles >24 months post-treatment (data not shown).
- Finally, at this long-term timepoint, immunofluorescence staining of treated muscle showed high-level SGCB expression and localization to sarcolemma (**Figure 4**) and no decrease of protein expression levels in all muscles (>95%) compared to earlier timepoints (**Figure 5**).

### Figure 4. Biomarker expression analysis: Immunofluorescence staining shows SGCB expression >24 months post-treatment




### Figure 5. Immunofluorescence staining shows no loss of SGCB expression over time




## 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, when delivered at an older age to more severely diseased muscle.
- These results also provide evidence on long-term durability of AAV gene therapy with no loss of SGCB expression that support long-term benefit clinically.

## ACKNOWLEDGEMENTS & DISCLOSURES

This study was sponsored by Sarepta Therapeutics, Inc. Authors are employees of Sarepta Therapeutics and may have stock options. Medical writing and editorial support was provided by Health & Wellness Partners, LLC, funded by Sarepta Therapeutics.

Presented at 25th International Annual Congress of the World Muscle Society| 30 Sep – 2 Oct, 2020 | Virtual Format