P.140

Systematic Gene Transfer with rAAVrh74.MHCK7.SGCB Increased β-sarcoglycan Expression in Patients with Limb Girdle Muscular Dystrophy Type 2E (LGMD2E)

Louise R. Rodino-Klapac,[1,3] Eric R. Pozsgai,[1,3] Sarah Lewis,[1,3] Danielle A. Griffin,[1,3] Aaron S. Meadows,[1,3] Kelly J. Lehman,[1]
Kathleen Church,[1] Natalie F. Miller,[1] Megan A. Iammarino,[1] Linda P. Lowes,[1] Jerry R. Mendell[1,2]

[1]Center for Gene Therapy, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio, USA; [2]Department of Pediatrics and Neurology, The Ohio State University, Columbus, OH, USA; [3]Sarepta Therapeutics, Inc., Cambridge, MA, USA

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 2E (LGMD2E) is caused by mutations in the β-sarcoglycan (SGCB) gene that result in loss of functional protein affecting other structural components of the dystrophin-associated protein complex.[2]
- LGMD2E usually manifests with progressive hip/shoulder muscle weakness and often includes cardiac involvement and elevated creatine kinase (CK).[3]
- There are currently no approved disease-modifying therapies for LGMD2E.
- 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.hSGCB construct was designed to restore functional SGCB to muscles (**Figure 1**).
- Long-term durability has been demonstrated in preclinical models for up to 8 years after treatment.[4]
- In this communication, we report for first time 18-month functional data for patients enrolled in the SRP9003-101 clinical gene transfer trial, Cohort 1, that supports the observation of long-term durability in patients and 6-month functional data for patients enrolled in Cohort 2.

Figure 1. rAAVrh74.MHCK7.hSGCB construct for the treatment of LGMD2E (β-sarcoglycanopathy)

Characteristic	Driven By	Expectations Based on Pre-clinical and Clinical Studies
Transduction	AAVrh74 Vector	AAVrh74 efficient transduction to muscles[5-7]
Expression	MHCK7 Promoter	MHCK7 selective for cardiac and skeletal transgene muscle expression[6-8] Widespread SGCB expression in all muscles[6,7]
Efficacy	SGCB Transgene	Reduction in CK[6,7] Improved functional outcomes[6,7]
Safety	AAVrh74 Vector and SGCB Transgene	Favorable safety profile[6,7]

ITR= inverted terminal repeats; pA= polyadenylation

OBJECTIVE

- To report the initial findings of the first-in-human, single-center, open-label systemic gene delivery, Phase I/II clinical gene transfer trial delivering rAAVrh74.MHCK7.hSGCB (SRP-9003) to patients with LGMD2E.

METHODS

Trial Design

- First-in-human, single-center, open-label, systemic gene delivery, Phase I/II study (NCT03652259).

rAAVrh74.MHCK7.hSGCB Construct

- Self-complementary (sc) adeno-associated virus vector, scAAVrh74, containing a codon-optimized full-length human SGCB transgene driven by a muscle-specific promoter (MHCK7; **Figure 1**).

Patient Population

- Eligible patients are aged 4-15 years with confirmed SGCB mutation (both alleles) who were negative for antibodies against AAVrh74 and scored ≥40% of normal on 100-meter timed test (**Table 1**).

Table 1. Demographic information for all 6 patients

Cohort/ Dose	Patient	Age (years)	Mutation	Weight (kg)	CK Levels at Baseline (U/L)
Cohort 1: $5x10^{13}$ vg/kg	1	13	Exon 3	57.2	10,727
	2	4	Exon 4	17.5	12,286
	3	13	Exon 3	50.4	10,985
Cohort 2: $2x10^{14}$ vg/kg	4	11	Exon 4	29.1	6,320
	5	11	Exon 3	39.5	8,938
	6	8	Exon 1	26.6	5,743

Treatment

- Patients in Cohort 1 were treated with systemic delivery of SRP-9003 in a peripheral vein at a dose of $5x10^{13}$ vg/kg.† Muscle biopsies at 8 weeks determined dosage level for Cohort 2 to be $2x10^{14}$ vg/kg.‡
- Patients in Cohort 2 received SRP-9003 systemic gene delivery at least 4 weeks post-biopsy of Cohort 1.
- SRP9003 was infused over approximately 1-2 hours.
- Prednisone 1 mg/kg/day was initiated to dampen the host immune response to AAV therapy 1 day before treatment, tapering after 60 days.

†The dose of $5x10^{13}$ vg/kg (supercoiled qPCR) or the linear qPCR equivalent of $1.85x10^{13}$ vg/kg was selected based on nonclinical data
‡The dose of $2x10^{14}$ vg/kg (supercoiled qPCR) or the linear qPCR equivalent of $7.41x10^{13}$ vg/kg (~4-fold increase over Cohort 1 dose) was selected based on nonclinical safety and tolerability data with SRP-9003

METHODS (CONT'D)

Primary Endpoint

- Safety

Secondary Endpoint

- Change in SGCB expression from baseline to week 8[§]

Other Endpoints

- Decrease in CK
- Functional endpoints: North Star Assessment of Limb-girdle Muscular Dystrophies[||] (NSAD) and timed functional tests (100-meter walk/run [100MWR], 10-meter walk/run [10MWR], 4-Stair Climb, and Time to Rise)

[§]Based on pre-clinical studies, the goal was to achieve expression levels of ≥20%
[||]Origin from North Star Assessment for Dysferlinopathy

RESULTS

Expression

- Robust full-length SGCB expression in muscle biopsies was observed by immunofluorescence (IF) and Western blot in all 6 patients at Day 60 post-treatment (**Figure 2, Table 2**).
- SGCB expression is supported at Day 60 by vector genome counts at both doses (**Table 2**) and upregulates α-sarcoglycan expression suggesting restoration of sarcoglycan complex to the membrane (data non shown).
- CK levels are reduced at Day 90 post-treatment with systemic SGCB gene therapy (**Table 2**).

Figure 2. Robust SGCB protein expression at the sarcolemma of skeletal muscle at Day 60 after systemic treatment with SRP-9003

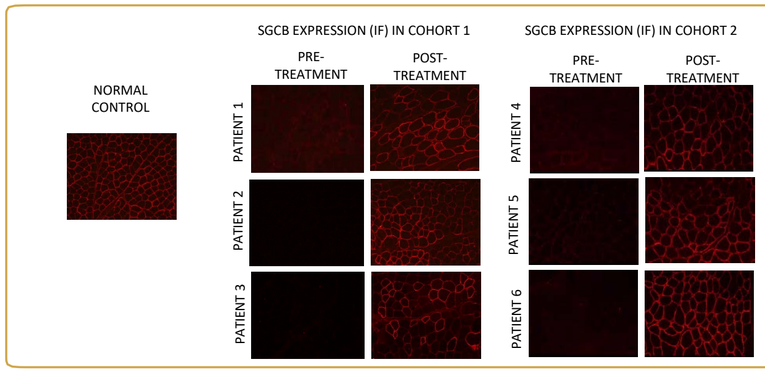


Table 2. Summary of SGCB expression and CK outcomes

	Vector Copies per Nucleus	% of SGCB-Positive Fibers (% NC)	Intensity (IF) (% NC)	Western Blot (% NC)	Change in CK Levels from BL
Mean Cohort 1	0.60	51%	47%	36.1%	-83.4%
Mean Cohort 2	4.2	72%	73%	62.1%	-89.1%

Functional Outcomes

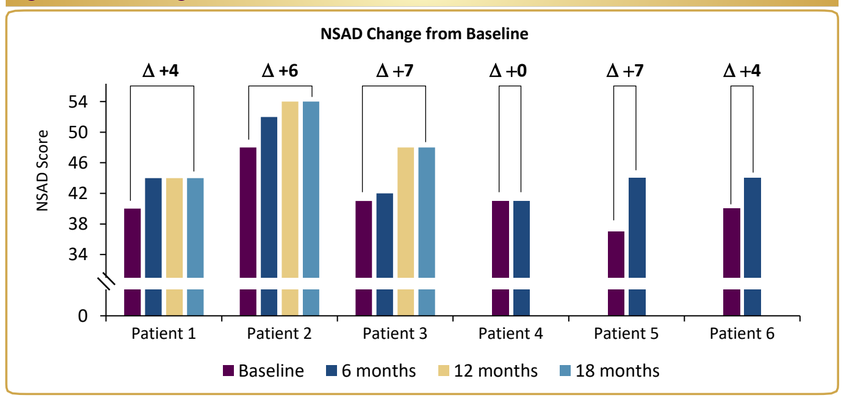
- Mean change from baseline in NSAD and timed functional tests for Cohort 1 and 2 are shown in **Table 3**.
- Recognizing limitations of small sample size and variability in clinical outcome measures, over the first 6 months, Cohort 2 demonstrated similar to improved functional gains compared with Cohort 1, with superior gains most evident in NSAD (**Figure 3**), 100MWR, and Time to Rise.

Table 3. Summary of functional outcomes clinical data at 6 months and 18 months

Functional Outcome Measure	Cohort 1	Cohort 2	Cohort 1
	Mean change from BL at 6 months	Mean change from BL at 6 months	Mean change from BL at 18 months
NSAD	+3.0	+3.7	+5.7
Time to Rise (sec)	-0.1	-1.3	-0.2
4-Stair Climb (sec)	-0.5	-0.4	-0.4
100MWR (sec)	-3.8	-6.3	-4.4
10MWR (sec)	-0.6	-0.6	-0.5

RESULTS (CONT'D)

Figure 3. NSAD change from baseline in both cohorts



Safety

Cohort 1 as July 8, 2020 (n=3)

- 2 patients had elevated liver enzymes, 1 of which was designated an SAE, as the patient had associated transient increase in bilirubin
  - 1 event occurred when the patient was tapered off oral steroids, the other occurred while the patient was being tapered
  - Returned to baseline and symptoms resolved within days following supplemental steroid treatment
- 1 patient experienced mild vomiting which resolved within 1 day without treatment
- No other clinically significant laboratory findings
  - No decreases in platelet counts observed outside of the normal range
  - No clinical sequelae associated with complement activation

Cohort 2 as July 8, 2020 (n=3)

- Majority of AEs were mild to moderate (eg, vomiting, pain in extremity), which resolved
- 1 treatment-related SAE observed
  - Dehydration resulting from vomiting 3 days after infusion, which resolved within 2 days with ondansetron, promethazine, and IV fluids
- 1 patient had mildly elevated GGT
  - Returned to within normal limits while on tapering dose of steroids; the patient did not experience an increase after tapering was concluded
- No stopping/discontinuation rules were triggered by AEs
- No other clinically significant laboratory findings
  - No decreases in platelet counts observed outside of the normal range
  - No clinical sequelae associated with complement activation

CONCLUSIONS

- Systemic administration of rAAVrh74.MHCK7.hSGCB (SRP-9003) is well tolerated; no unexpected immunological responses have been observed
- Results show an efficient transduction and robust SGCB protein expression in all patients post-infusion with SRP-9003, which resulted in the re-constitution of the sarcoglycan complex and reductions in CK
- 6 months post-infusion with SRP-9003, patients experienced improvements in functional measures compared to baseline
- A durable response in functional outcomes is observed out to 18 months in Cohort 1, with improvements in NSAD and timed tests over baseline values
- Together, these data suggest the long-term efficacy of the SRP-9003 gene transfer therapy, supporting continued development of the high dose, and providing evidence to advance the clinical development program

REFERENCES

1. Liewluck T, Milone M. *Muscle Nerve*. 2019;58(2):167-177.
2. McNally EM. *The Sarcoglycans*. Landes Bioscience. 2000-2013.
3. Taghizadeh E, et al. *J Cell Physiol.* 2018;234(6):7874-7884.
4. Li J, et al. *Mol Ther*. 2016;24(suppl 1):S284.
5. Chicoine LG, et al. *Mol Ther*. 2014;22(4):713-724.
6. Mendell JR, et al. *JAMA Neurol*. 2020;e201484.
7. Pozsgai ER, et al. *Mol Ther.* 2017;25(4):855-869.
8. Salva MZ, et al. *Mol Ther.* 2007;15(2):320-329.

ACKNOWLEDGEMENTS & DISCLOSURES

This study was sponsored by Sarepta Therapeutics, Inc. (NCT03652259). rAAVrh74.MHCK7.hSGCB is an investigational therapy and has not been reviewed or approved by the FDA or EMA. L.R. Rodino-Klapac, E.R. Pozsgai, S. Lewis, D.A. Griffin, and A.S. Meadows are employees of Sarepta Therapeutics, Inc. and may have stock options. J.R. Mendell received financial support from Sarepta Therapeutics, Inc. for the travel and accommodation costs of study participants. K.J. Lehman, K. Church, N.F. Miller, M.A. Iammarino, and L.P. Lowes have no conflicts to disclose. Medical writing and editorial support was provided by Health & Wellness Partners, LLC, Upper Saddle River, New Jersey, funded by Sarepta Therapeutics, Inc.