Safety, β-Sarcoglycan Expression, and Functional Outcomes From Systemic Gene Transfer of rAAVrh74.MHCK7.hSGCB in LGMD2E/R4

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Disclosures

- LRR-K, ERP, SL, DAG, ASM, SN, and XL are or have been employees of Sarepta Therapeutics, Inc., and may have stock options
- JRM received financial support from Sarepta Therapeutics, Inc., for the travel and accommodation costs of study participants
- KJL, KC, NFR, MAI, and BS have no conflicts to disclose
- LNA and LPL received fees from Sarepta for licensure of the natural history data set

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- rAAVrh74.MHCK7.hSGCB (SRP-9003) is an investigational therapy and has not been reviewed or approved by the FDA or EMA

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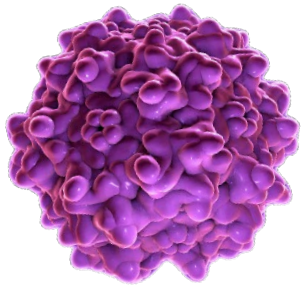
SRP-9003: Investigational gene therapy for limb-girdle muscular dystrophy type 2E/R4 (LGMD2E/R4)

- LGMD2E/R4 is caused by mutations in the *β-sarcoglycan (SGCB)* gene[1,2]
- Adeno-associated virus (AAV)–mediated gene transfer therapy to express full-length SGCB has the potential to treat LGMD2E/R4

SRP-9003: Self-complementary AAV vector

rAAVrh74



*Efficient **skeletal and cardiac muscle transduction**[3]*

MHCK7 promoter

*Optimized for expression in **skeletal and cardiac muscle**[4-6]*

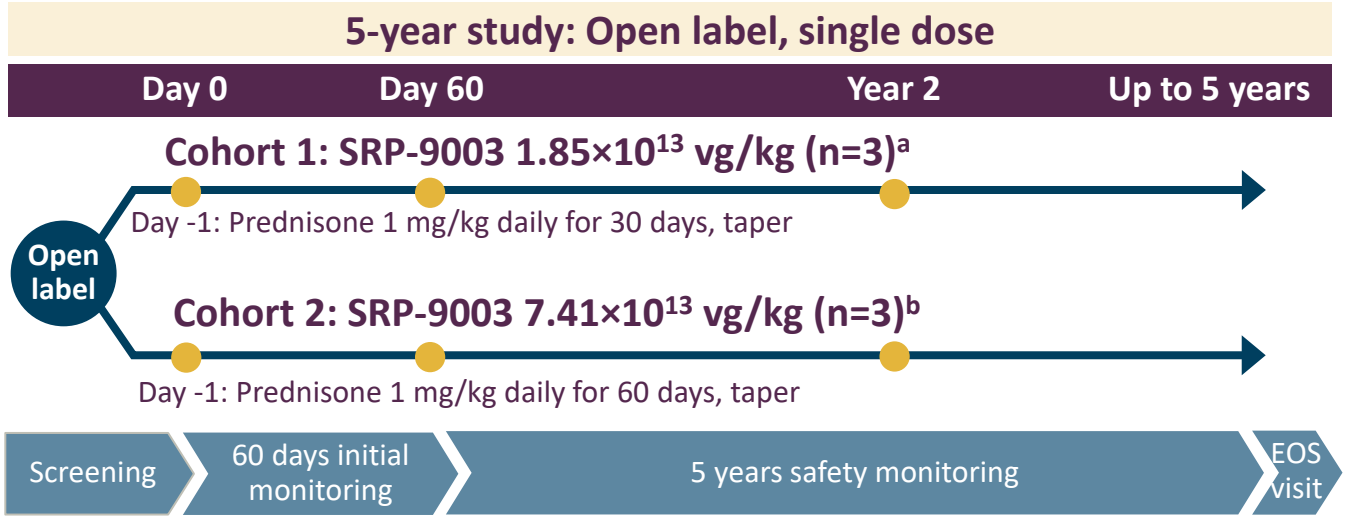
hSGCB cDNA

***Full-length human SGCB** gene transfer*

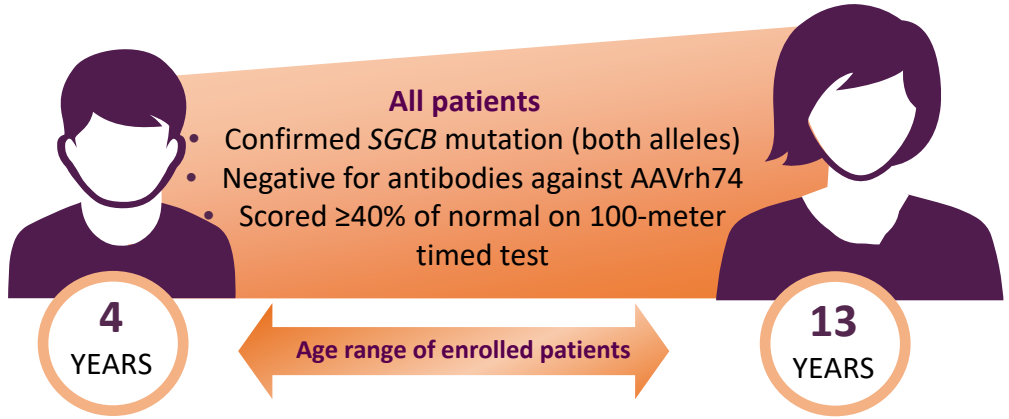
Objective: To report the interim findings of an ongoing phase 1/2 clinical gene transfer trial delivering SRP-9003 to patients with LGMD2E/R4 (Study SRP-9003-101; NCT03652259)

1. McNally EM. *The Sarcoglycans.* Austin, TX: Landes Bioscience. 2000-2013. 2. Gao Q, McNally EM. *Compr Physiol.* 2015;5:1223-39. 3. Chicoine LG, et al. *Mol Ther.* 2014;22:338-47. 4. Salva MZ, et al. *Mol Ther.* 2007;15:320-29.
5. Pozsgai ER, et al. *Mol Ther.* 2017;25:855-69. 6. Wang B, et al. *Gene Ther.* 2008;15:1489-99.

Study design: First-in-human, open-label, phase 1/2 study



- **Primary endpoint:** Safety
- **Secondary endpoint:** SGCB expression at week 8
- **Other endpoints**
  - Change in creatine kinase (CK) from baseline
  - Functional endpoints (NSAD and timed tests: 100m, 10m, 4-stair climb, and time to rise)



Baseline characteristics

Cohort	Patient	Age, years	Mutation	Weight, kg	CK, U/L
Cohort 1	1	13	Exon 3[c]	57.2	10,727
	2	4	Exon 4[c]	17.5	12,286
	3	13	Exon 3[c]	50.4	10,985
Cohort 2	4	11	Exon 4[c]	29.1	6320
	5	11	Exon 3[c]	39.5	8938
	6	8	Exon 1[d]	26.6	5743

[a]1.85×10[13] vg/kg (linear qPCR; 5×10[13] vg/kg supercoiled qPCR equivalent); [b]7.41×10[13] vg/kg (linear qPCR; 2×10[14] vg/kg supercoiled qPCR equivalent); [c]Missense mutation; [d]Nonsense mutation.
10m=10-m timed test; 100m=100-m timed test; EOS=end of study; NSAD=North Star Assessment for Limb-girdle Type Muscular Dystrophies; qPCR=quantitative polymerase chain reaction; SGCB=β-sarcoglycan; vg=vector genome copies.

Safety results: Most common treatment-related adverse events (TEAEs)

System organ class preferred term	Cohort 1 (n=3) n (%)	Cohort 2 (n=3) n (%)	Total (N=6) n (%)
Patients with any treatment-related TEAEs[a]	2 (66.7)	3 (100.0)	5 (83.3)
Gastrointestinal disorders	1 (33.3)	3 (100.0)	4 (66.7)
Abdominal pain	0	2 (66.7)	2 (33.3)
Abdominal pain upper	1 (33.3)	1 (33.3)	2 (33.3)
Nausea	0	2 (66.7)	2 (33.3)
Vomiting	1 (33.3)	3 (100.0)	4 (66.7)
General disorders and administration-site conditions	0	1 (33.3)	1 (16.7)
Pyrexia	0	1 (33.3)	1 (16.7)
Hepatobiliary disorders	1 (33.3)	0	1 (16.7)
Hepatitis	1 (33.3)	0	1 (16.7)
Hyperbilirubinemia	1 (33.3)	0	1 (16.7)
Investigations	2 (66.7)	3 (100.0)	5 (83.3)
Gamma-glutamyl transferase increased	2 (66.7)	1 (33.3)	3 (50.0)
Neutrophil count decreased	0	1 (33.3)	1 (16.7)
White blood cell count decreased	0	2 (66.7)	2 (33.3)
Metabolism and nutrition disorders	1 (33.3)	1 (33.3)	2 (33.3)
Decreased appetite	1 (33.3)	0	1 (16.7)
Dehydration	0	1 (33.3)	1 (16.7)
Nervous system disorders	1 (33.3)	0	1 (16.7)
Dizziness	1 (33.3)	0	1 (16.7)

[a]TEAEs are defined as all AEs (as of January 18, 2022) that started on or after the study drug administration date. AEs are coded using MedDRA version 22.0.
MedDRA=Medical Dictionary for Regulatory Activities.

Results reinforce acceptable safety profile, with no new safety signals

Cohort 1 as of January 18, 2022 (n=3)

- 2 patients had elevated liver enzymes; 1 instance was designated a serious AE (SAE) and associated with transient increase in bilirubin
  - Occurred during or after steroid tapering; resolved within days following supplemental steroid treatment
- 1 patient experienced mild vomiting that resolved within 1 day without treatment

Cohort 2 as of January 18, 2022 (n=2)

- Majority of AEs were mild to moderate (eg, vomiting, pain in extremity) and resolved
- 1 treatment-related SAE was observed
  - Dehydration resulting from vomiting 3 days after infusion; resolved within 2 days with treatment
- 1 patient had mildly elevated GGT
  - Returned to within normal limits while on tapering dose of steroids; GGT levels did not increase after steroid treatment
- One of the participants in this trial died due to a recreational accident unrelated to the study

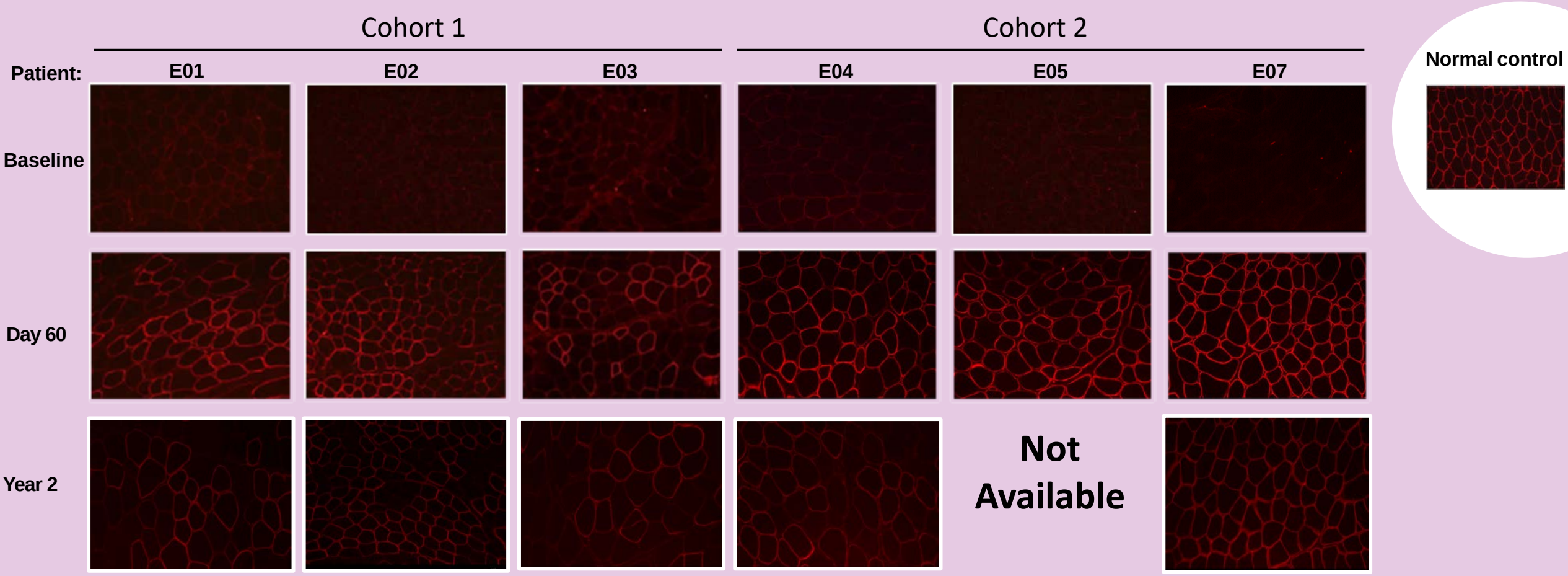
Both cohorts

- Most common treatment-related AEs were vomiting (4 subjects) and GGT elevation (3 subjects)
- No stopping/discontinuation rules were triggered by AEs
- No other laboratory abnormalities were suggestive of safety concerns
  - No decreases in platelet counts observed outside the normal range
  - No clinical sequelae associated with complement activation

Results show no new safety signals, and treatment-related AEs occurred early and were transient and manageable

AE=adverse event; GGT=gamma-glutamyl transferase.

SGCB expression: Robust expression and sarcolemmal localization of SGCB at day 60 post infusion



SGCB=β-sarcoglycan.

SGCB expression at 60 days post infusion was sustained for 2 years in both Cohorts

Cohort 1 ($1.85x10^{13}$ vg/kg[a])

Timepoint	Western blot (% NC)	% SGCB+ fibers (% NC)	SGCB intensity (% NC)	Vector copies per nucleus (ddPCR)[c]
Day 60 n=3	36 (2.7)	51 (10.6)	47 (9.5)	---
Year 2 n=3	54 (16.1)	47 (21.3)	35 (22.9)	0.46 (0.4)

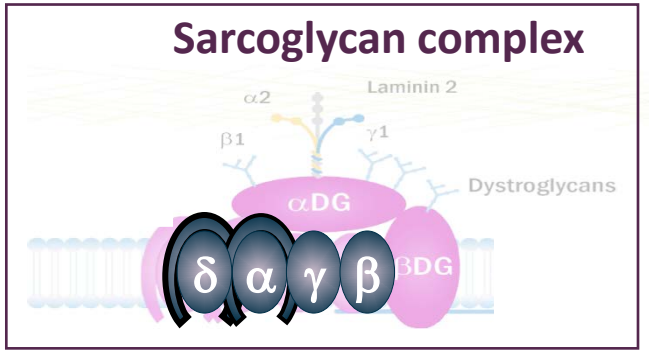
Cohort 2 ($7.41x10^{13}$ vg/kg[b])

Timepoint	Western blot (% NC)	% SGCB+ fibers (% NC)	SGCB intensity (% NC)	Vector copies per nucleus (ddPCR)
Day 60 n=3	62 (8.7)	72 (6.2)	73 (21.8)	2.26 (0.9)
Year 2 n=2[d]	60 (21.4)	63 (21.6)	44 (33.2)	0.52 (0.3)

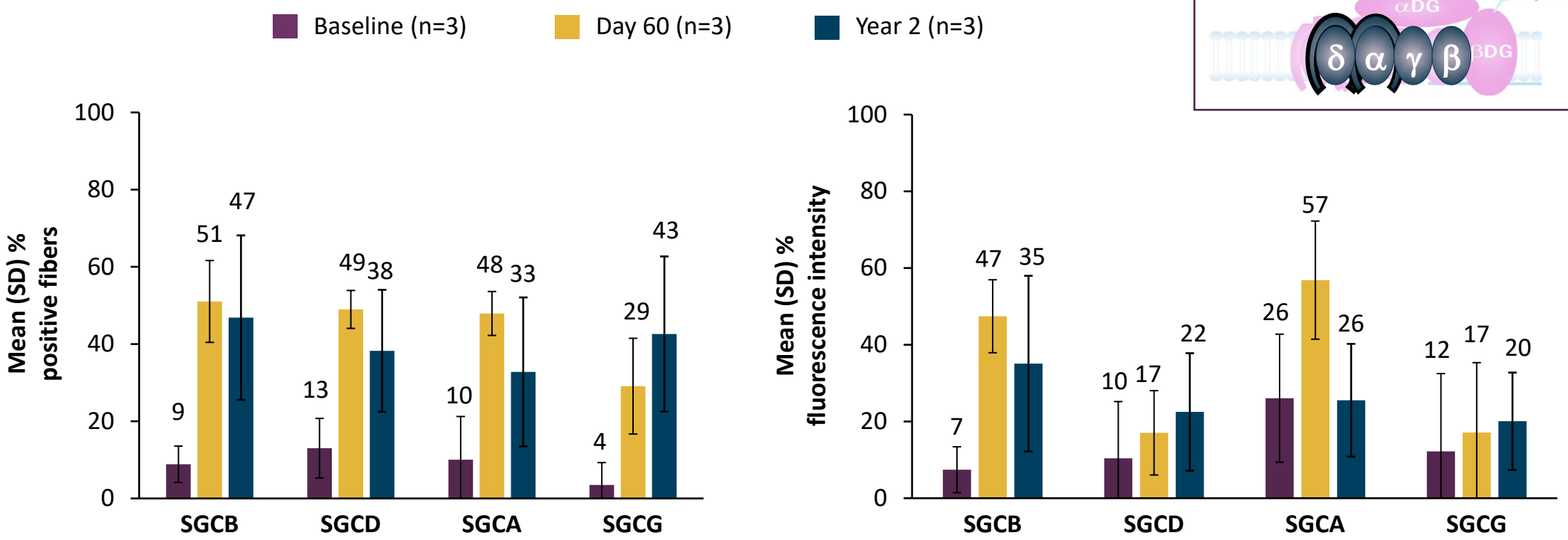
A dose response in full-length SGCB protein expression was observed at day 60 and sustained at 2 years

Values are mean (SD). [a]$1.85×10^{13}$ vg/kg (linear qPCR; $5×10^{13}$ vg/kg supercoiled qPCR equivalent); [b]$7.41×10^{13}$ vg/kg (linear qPCR; $2×10^{14}$ vg/kg supercoiled qPCR equivalent); [c]Mean (SD) qPCR value of day 60 Cohort 1 was 0.59 (0.4); [d]Cohort 2 year 2, n=2.
ddPCR=droplet digital PCR; IF=immunofluorescence; NC=normal control; PCR=polymerase chain reaction; qPCR=quantitative PCR; SGCB=β-sarcoglycan.

Cohort 1: SGCB expression results in reconstitution of the sarcoglycan complex up to year 2

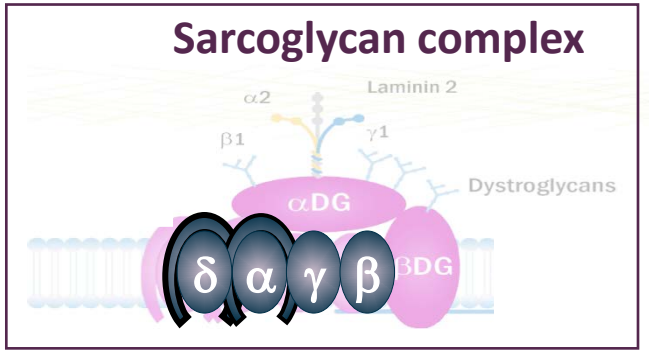


Expression of the sarcoglycan complex by immunofluorescence

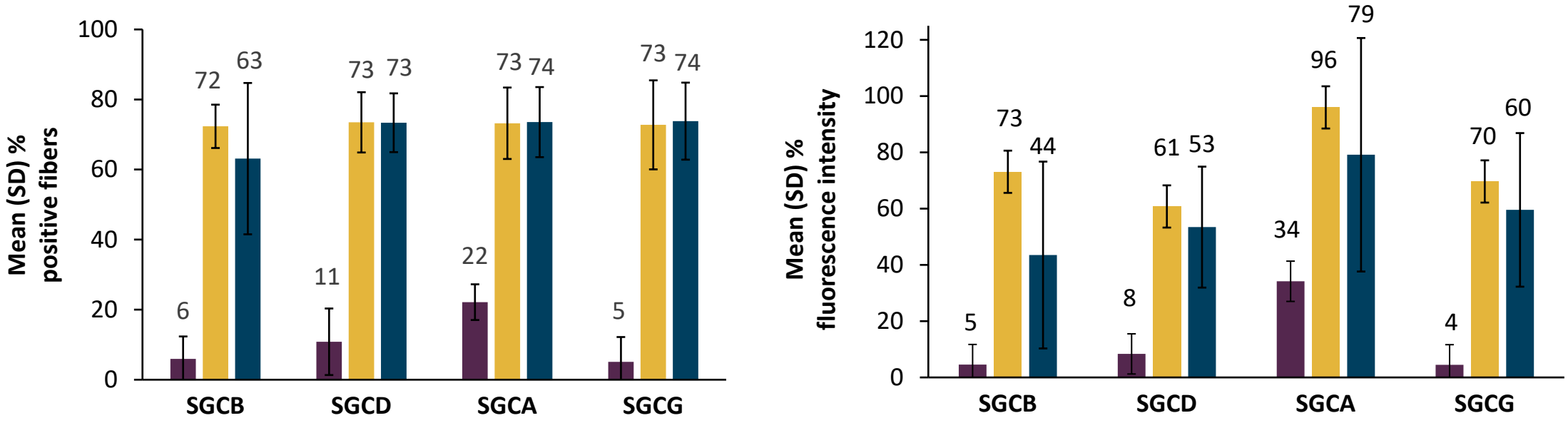


SGCA=α-sarcoglycan; SGCB=β-sarcoglycan; SGCD=δ-sarcoglycan; SGCG=γ-sarcoglycan.

Cohort 2: SGCB expression results in reconstitution of the sarcoglycan complex up to year 2

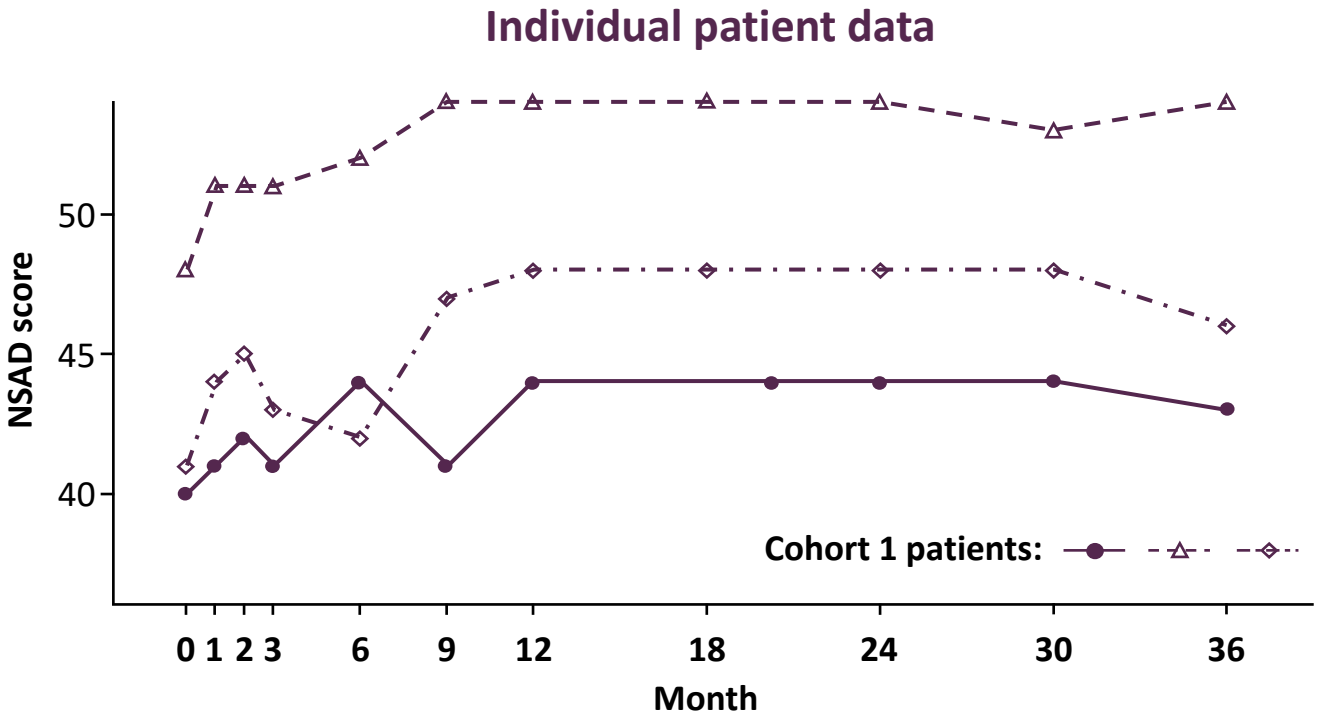


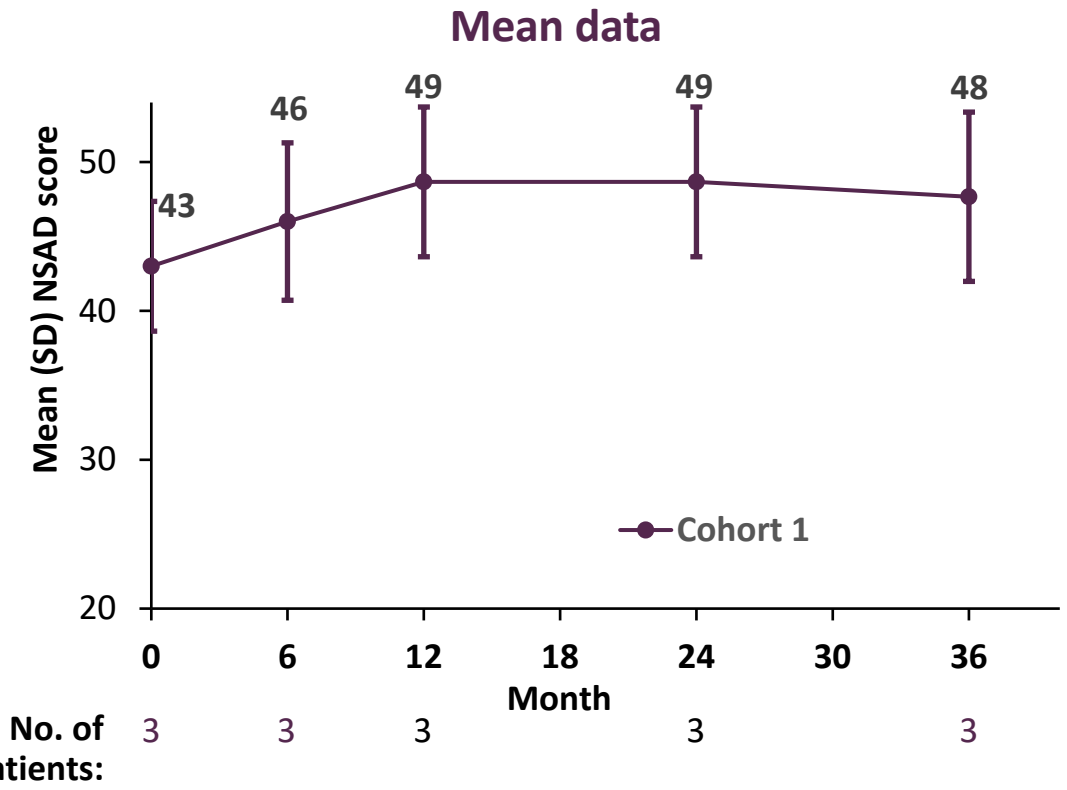
Expression of the sarcoglycan complex by immunofluorescence



SGCA=α-sarcoglycan; SGCB=β-sarcoglycan; SGCD=δ-sarcoglycan; SGCG=γ-sarcoglycan.

Cohort 1 functional outcomes: SRP-9003 treatment resulted in persistent improvement in NSAD total score up to 3 years



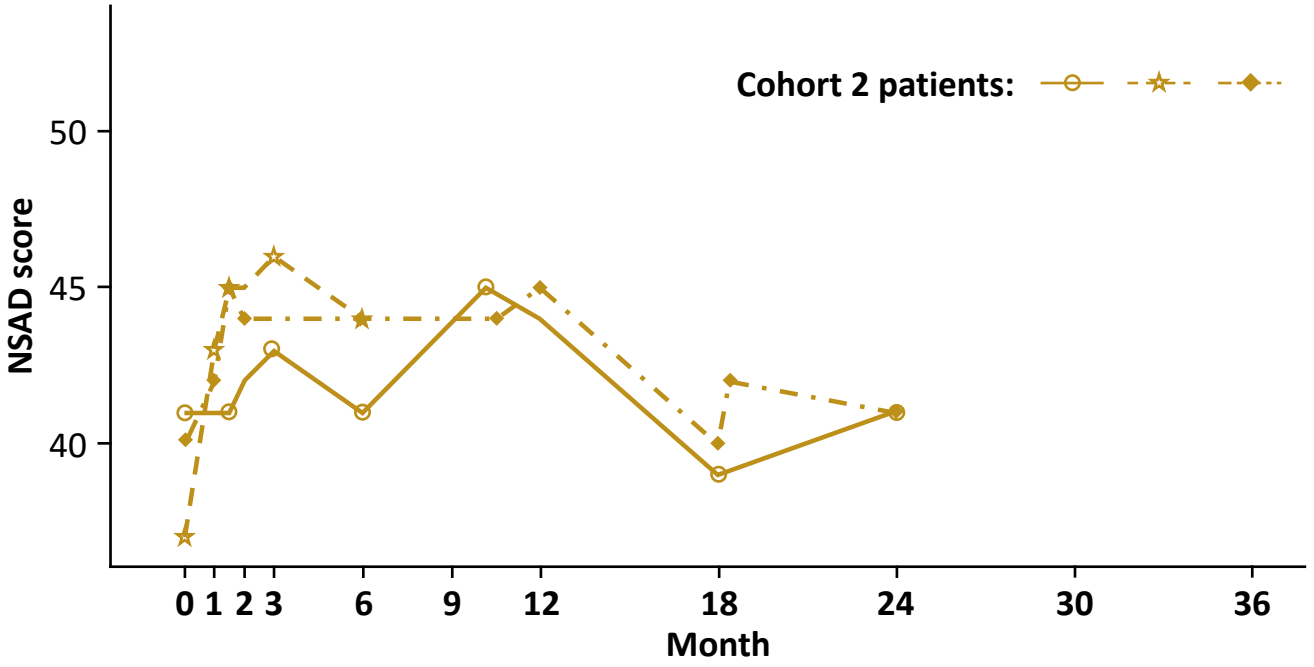


Patients treated with SRP-9003 in Cohort 1 demonstrated improvements in NSAD that were sustained for 3 years

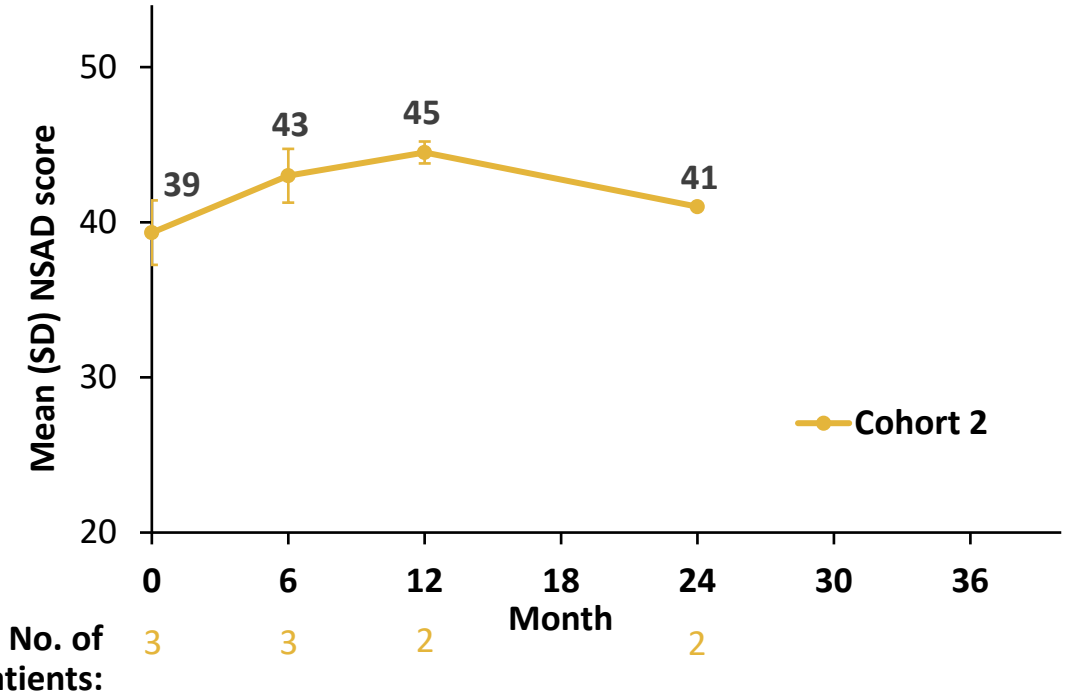
NSAD=North Star Assessment for Limb-girdle Type Muscular Dystrophies.

Cohort 2 functional outcomes: SRP-9003 treatment resulted in persistent stabilization at or over baseline in NSAD total score for 2 years

Individual patient data



Mean data



Patients treated with SRP-9003 in Cohort 2 demonstrated stabilization at or over baseline in NSAD that was sustained for 2 years

NSAD=North Star Assessment for Limb-girdle Type Muscular Dystrophies.

SRP-9003 treatment resulted in sustained improvements in timed function tests

Mean (SD) change from baseline (s)	Cohort 1 (1.85×10^{13} vg/kg)[a]				Cohort 2 (7.41×10^{13} vg/kg)[b]		
	6 months n=3	12 months n=3	24 months n=3	36 months n=3	6 months n=3	12 months n=2	24 months n=2
Time to rise	−0.2 (0.8)	−0.8 (0.4)	−0.6 (0.2)	−0.3 (0.3)	−1.3 (0.9)	−1.1 (1.1)	−0.7 (0.4)
4-stair climb	−0.5 (0.4)	−0.5 (0.3)	−0.3 (0.4)	−0.2 (0.6)	−0.4 (0.3)	−0.4 (0.0)	−0.3 (0.3)
100m	−3.8 (2.9)	−5.3 (3.2)	−2.8 (6.4)	+2.6 (13.0)	−6.3 (6.7)	−7.9 (5.4)	−2.9 (9.7)
10m	−0.6 (0.3)	−0.6 (0.2)	−0.2 (0.5)	0 (0.9)	−0.6 (0.6)	−0.6 (0.2)	−0.3 (0.9)

Negative numbers correspond to faster test times.

Patients treated with SRP-9003 demonstrated improvements over baseline in timed function tests, which were generally sustained for 3 years in Cohort 1 and 2 years in Cohort 2

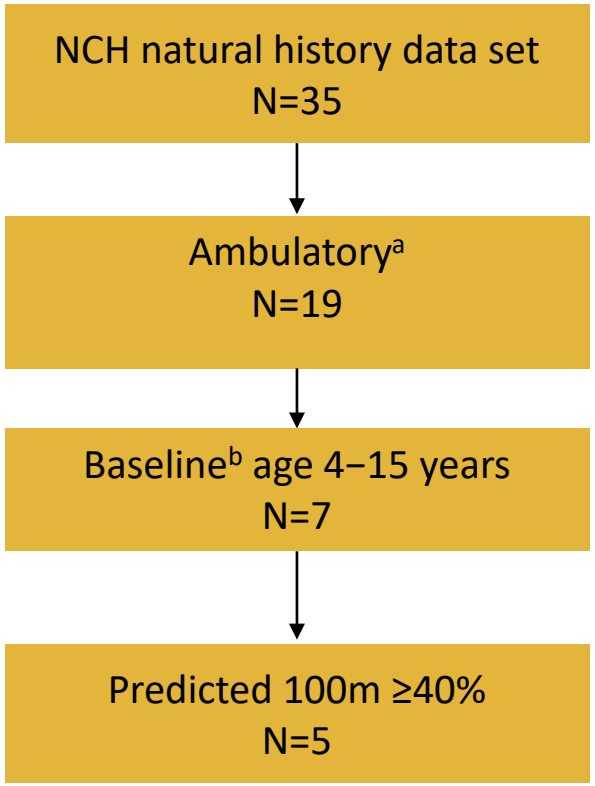
[a] 1.85×10^{13} vg/kg measured using linear reference plasmid DNA quantitative polymerase chain reaction (qPCR), supercoiled reference DNA equivalent is 5×10^{13} vg/kg;
[b] 7.41×10^{13} vg/kg measured using linear reference plasmid DNA qPCR, supercoiled reference DNA equivalent is 2×10^{14} vg/kg. 10m=10-m timed test; 100m=100-m timed test.

Selection of natural history control cohort for comparison with SRP-9003-101

Selected comparison cohort from Nationwide Children's Hospital (NCH) natural history data set, based on the same key inclusion criteria as in Study SRP-9003-101

- Ambulatory status (yes)
- Baseline age (4–15 years)
- Predicted 100m (≥40%)

NCH LGMD2E/R4 natural history cohort



[a]Ambulatory defined as presence of 10m timed test value; [b]Baseline was defined as the first time point where both 100m and NSAD were nonmissing.
100m=100-m timed test; LGMD2E/R4=limb-girdle muscular dystrophy type 2E/R4; NSAD=North Star Assessment for Limb-girdle Type Muscular Dystrophies.

Baseline comparison of SRP-9003–treated patients vs natural history cohort

Baseline characteristics comparison

	SRP-9003-101 (N=6)	NCH (N=5)
Age (years)	10.0 (3.5)	9.8 (3.2)
Male, n (%)	3 (50)	3 (60)
NSAD score	41.2 (3.7)	49.0 (3.9)
100m (s)	51.4 (10.5)	38.9 (3.9)
10m (s)	5.1 (0.9)	4.4 (0.3)[a]

Values are mean (SD) unless noted otherwise.

Age and sex are well balanced between 9003-101 patients and the NCH control cohort

Baseline functional endpoints scores are ***higher*** in the NCH control cohort

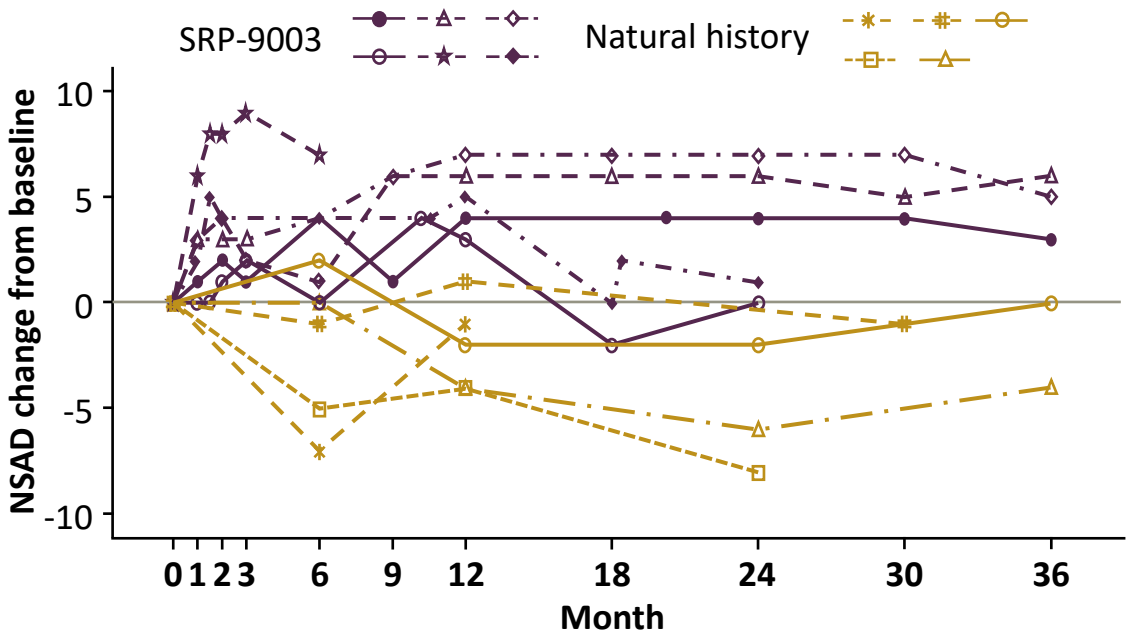
Explored alternative matching criteria; however, still unable to achieve balanced baseline functions

Therefore, used mixed-model repeated measures analysis to adjust for baseline function

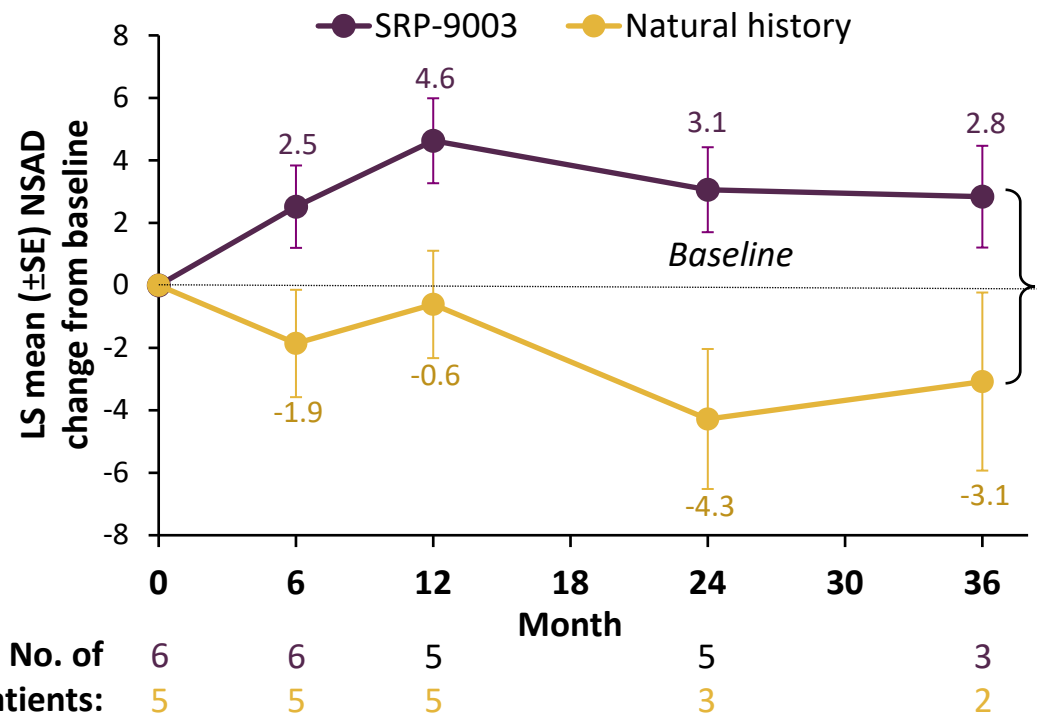
[a]N=4.
10m=10-m timed test; 100m=100-m timed test; NCH=Nationwide Children's Hospital; NSAD=North Star Assessment for Limb-girdle Type Muscular Dystrophies.

SRP-9003–treated patients display an improvement in total NSAD score vs natural history

NSAD change from baseline: Individual patient data



NSAD change from baseline: LS means by cohort



LS mean change at year 3: 5.9 points (95% CI, -1.5, 13.3)

Patients treated with SRP-9003 demonstrated clinically meaningful improvements in functional outcomes in an exploratory comparison vs an LGMD2E/R4 natural history cohort, as measured by NSAD

MMRM analysis included fixed effects for treatment arm, visit, and treatment arm by visit interaction, and baseline NSAD, baseline 100m, and baseline 10m as continuous covariates; the first-order autoregressive structure was used for variance-covariance matrix of within-patient errors. LGMD2E/R4=limb-girdle muscular dystrophy type 2E/R4; LS=least squares; MMRM=mixed-model repeated measures; NSAD=North Star Assessment for Limb-girdle Type Muscular Dystrophies.

SRP-9003-101: Summary

	1	2	3	4	5
QUESTION[1]	What was the safety and tolerability experience with SRP-9003?	Is the transgene DNA inside muscle cells?	Is the desired protein made?	Is the protein at the cell membrane?	Is muscle function improved?
EXPERIMENT	SAFETY	VECTOR GENOME COPIES/NUCLEUS	WESTERN BLOT	IMMUNOFLUORESCENCE	FUNCTIONAL OUTCOMES

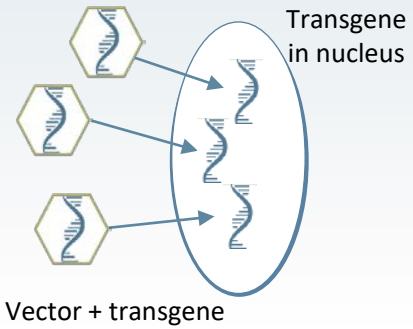
SAFETY

- Systemic administration of SRP-9003 is well tolerated to date with up to 3 years of follow-up for Cohort 1 and 2 years for Cohort 2
- No unexpected immunologic responses in these patients

VECTOR GENOME COPIES/NUCLEUS

At day 60:

- **C.1: 0.6** copies per nucleus
- **C.2: 4.2** copies per nucleus



WESTERN BLOT

SGCB expression

- **C.1: D60 36%; Y2 54%**
- **C.2: D60 62%; Y2 60%**

IMMUNOFLUORESCENCE

Percentage of cells with protein

Percentage of SGCB-positive fibers:

- **C.1: D60 51%; Y2 47%**
- **C.2: D60 72%; Y2 63%**

Intensity of fluorescent signal:

- **C.1: D60 47%; Y2 35%**
- **C.2: D60 73%; Y2 44%**

Rescue of SGCA, SGCG, and SGCD reconstitution of the sarcoglycan complex within the DAPC

FUNCTIONAL OUTCOMES

NSAD and TFTs

Mean (SD) NSAD score vs baseline (BL):

- **C.1:** 48 (5.7) Y3 vs 43 (4.4) BL
- **C.2:** 41 (0) Y2 vs 39 (2.1) BL

LS mean change from baseline of treated patients compared with natural history cohort at Y3:

- **5.2-point difference** (95% CI, -1.5, 13.3)

C.1=Cohort 1; C.2=Cohort 2; CK=creatine kinase; D=day; DAPC=dystrophin-associated protein complex; LS=least squares; NSAD=North Star Assessment of Limb-girdle type Muscular Dystrophies; SGCA=α-sarcoglycan; SGCB=β-sarcoglycan; SGCD=δ-sarcoglycan; SGCG=γ-sarcoglycan; TFT=timed function test; Y=year.

1. Asher DR, et al. *Expert Opin Biol Ther*. 2020;20:263-74.

Conclusions

- This interim analysis reinforces the acceptable safety profile of systemically administered SRP-9003
- SRP-9003 showed efficient transduction and drove robust, dose-dependent SGCB protein expression in all patients at day 60, resulting in reconstitution of the sarcoglycan complex; SGCB expression was sustained up to 2 years
- Patients treated with SRP-9003 demonstrated persistent stabilization at or over baseline in NSAD that were sustained up to 3 years in Cohort 1 and 2 years in Cohort 2; results were similar for timed function tests
- Exploratory post hoc analysis showed SRP-9003–treated patients had clinically important improvements in functional outcomes, as measured by NSAD, compared with a natural history cohort up to 3 years
- The observed durable treatment effect provides proof of concept and supports further clinical assessment of SRP-9003 gene transfer therapy in patients with LGMD2E/R4

Key Takeaway:
Persistence of SRP-9003 in transduced muscle continues to drive meaningful levels of SGCB expression over time, leading to sustained functional improvements

LGMD2E/R4=limb-girdle muscular dystrophy type 2E/R4; NSAD=North Star Assessment for Limb-girdle Type Muscular Dystrophies; SGCB=β-sarcoglycan.

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  - Lindsay N. Alfano
  - Megan A. Iammarino
  - Brenna Sabo
  - Natalie F. Reash
  - Kiana Shannon