In Situ Biodistribution and Localization of Bidridistrogene Xeboparvovec (SRP-9003) in LGMD2E/R4 Mice

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Disclosures

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Limb-girdle muscular dystrophy 2E/R4

- LGMD2E/R4 is caused by mutations in the ***SGCB*** gene
  - **Mutations cause loss of SGCB protein** and sarcoglycan components of the DAPC
- In patients, the loss of functional SGCB protein leads to progressive muscle degeneration and shortened lifespan[1–3]

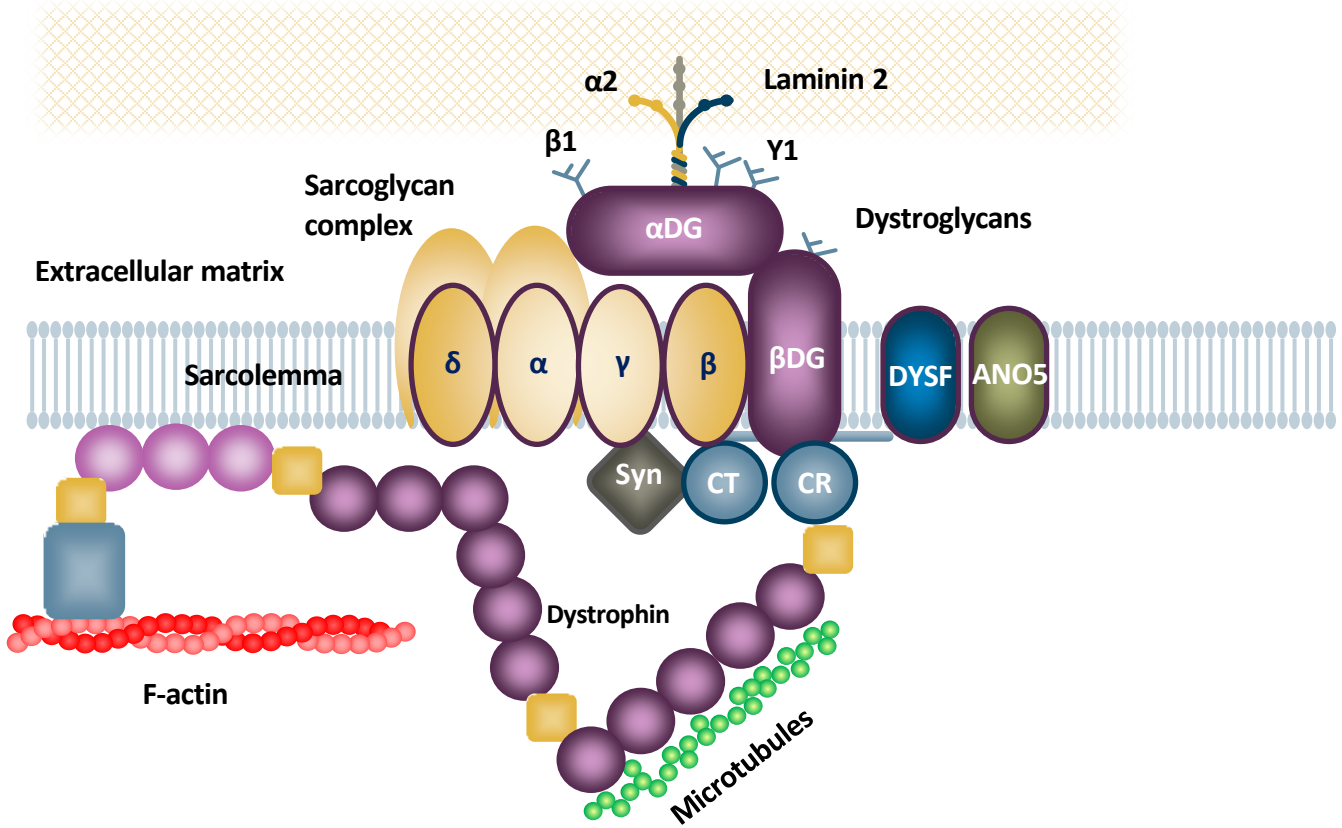
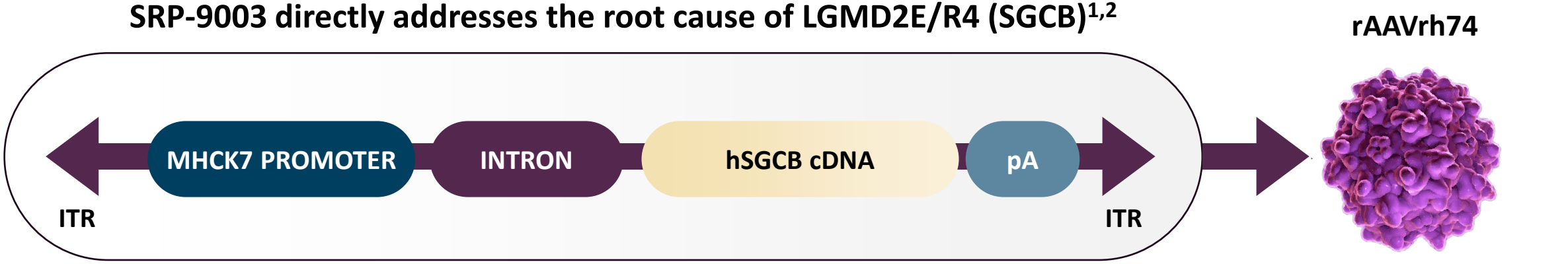


Image adapted from Fairclough RJ, et al. *Nat Rev Genet.* 2013;14(6):373-8 and Zhao J, et al. *Hum Mol Genet.* 2016;25(17):3647-53.
ANO5=anoctamin 5; CR=cysteine-rich domain; CT=c-terminus; DAPC=dystrophin-associated protein complex; DG=dystroglycan; DYSF=dysferlin; LGMD2E/R4=limb-girdle muscular dystrophy type 2E; SGCB=β-sarcoglycan; Syn=syntrophin.
1. https://rarediseases.info.nih.gov/diseases/6907/limb-girdle-muscular-dystrophy [Accessed Feb 5, 2024]. **2.** Murphy AP, Straub V. *J Neuromuscul Dis*. 2015;22:S7-19. **3.** Bouchard C, Tremblay JP. *J Clin Med*. 2023;12:4769.

SRP-9003 (bidridistrogene xeboparvovec)

SRP-9003 directly addresses the root cause of LGMD2E/R4 (SGCB)[1,2]



Optimized for expression in ***skeletal and cardiac muscle***[3–5]

Full-length hSGCB *gene transfer*

Efficient ***skeletal and cardiac muscle transduction***[6]

cDNA=complementary DNA; hSGCB=human β-sarcoglycan; ITR=inverted terminal repeat; LGMD2E/R4=limb-girdle muscular dystrophy type 2E; MHCK7=myosin heavy chain enhancer promoter; rAAVrh74=recombinant adeno-associated virus serotype rh74; pA=polyadenylation; SGCB=β-sarcoglycan.

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

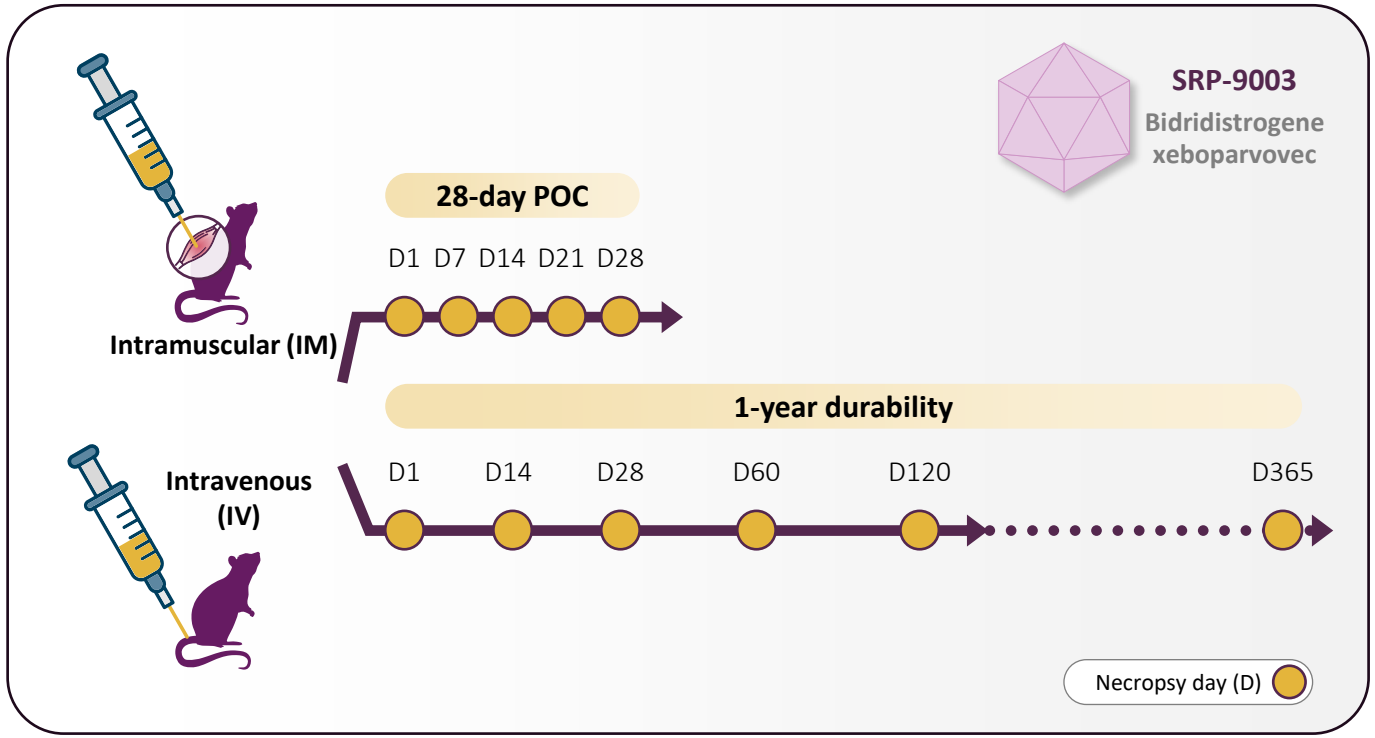
Study design and objective

For *IM* administration, necropsy timepoints were D1, D7, D14, D21, and D28

- **Objective:** POC for RNAscope™ assay
- SRP-9003 dose: 3×10^{10} total vg

For *IV* evaluation, necropsy timepoints were D1, D14, D28, D60, D120, and D365[a]

- **Objective:** Durability, spatial distribution
- SRP-9003 dose: 7.41×10^{13} vg/kg



Objective: Determine spatial distribution of SRP-9003 in situ

[a]D365 data have not been reached.
D=day; IM=intramuscular; IV=intravenous; POC=proof of concept; vg=vector genomes.

RESULTS

SRP-9003 – IM Administration

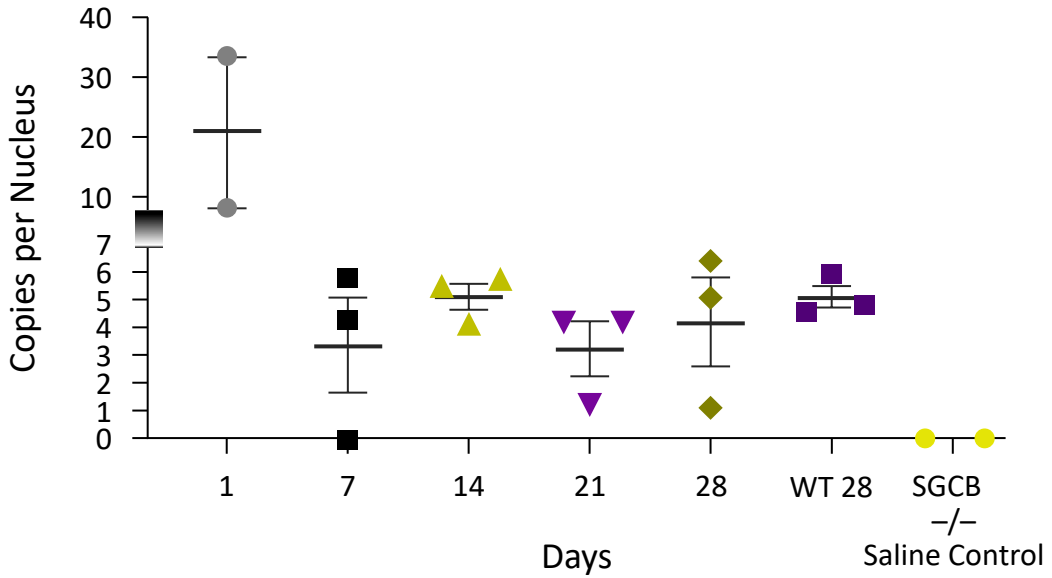
RESULTS

SRP-9003 biodistribution: transduction confirmation (IM)

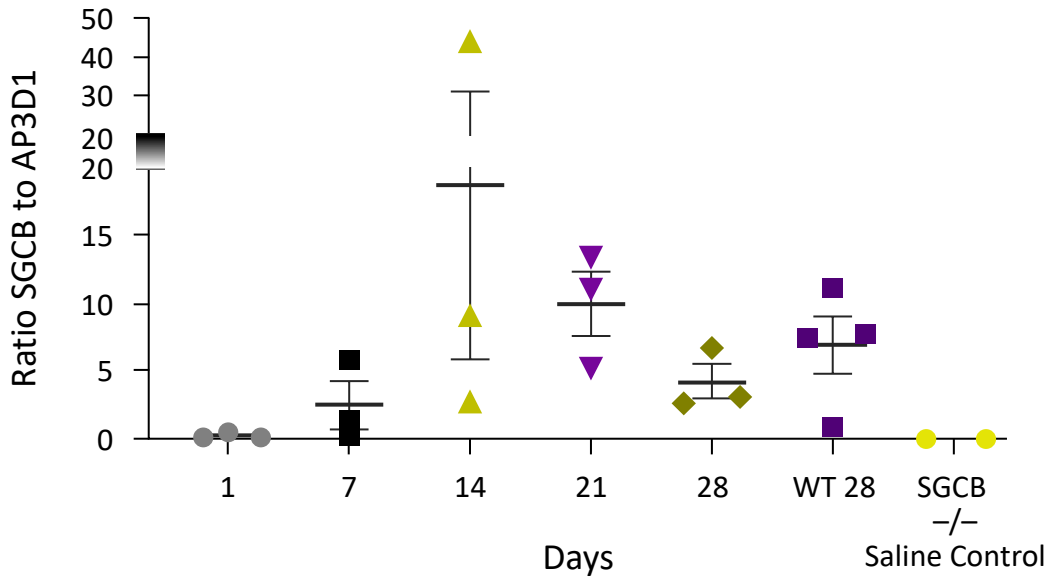
In TA muscle, SRP-9003 vector DNA **peaked on D1 and stabilized through D28** following IM SRP-9003 administration

In TA muscle, mRNA levels **were maximized on D14 and stabilized through D28** following IM SRP-9003 administration

SRP-9003 Vector DNA Levels (ddPCR)



SRP-9003 hSGCB mRNA Levels (RT-ddPCR)



Note: error bars represent standard error of the mean.

AP3D1=adaptor-related protein complex 3 subunit delta 1; D=day; ddPCR=droplet digital polymerase chain reaction; hSGCB=human β-sarcoglycan; IM=intramuscular; mRNA=messenger RNA; RT-ddPCR=reverse transcription droplet digital polymerase chain reaction; SGCB=β-sarcoglycan; TA=tibialis anterior; WT, wild type.

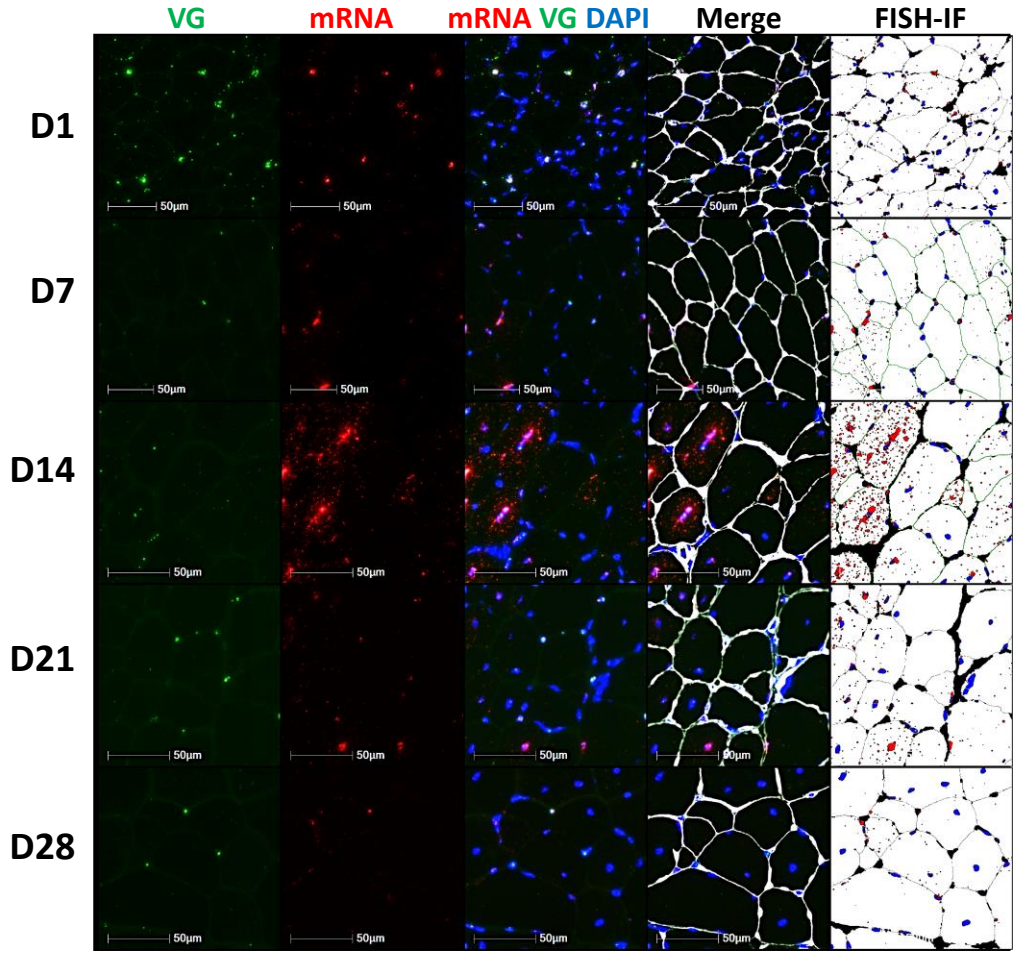
RESULTS

Detection of SRP-9003 in situ using RNAscope™ (IM)

- Robust **nuclear SRP-9003 transduction** was observed as early as **1 day post IM injection in skeletal muscle**, with SRP-9003 transgene mRNA levels peaking **14 days post IM injection**

Probe 1: Vector DNA (vg)
Probe 2: Transgene mRNA
Nuclear label: DAPI
Membrane marker: Laminin

SRP-9003 Detection in Muscle Post IM Injection Using RNAscope™

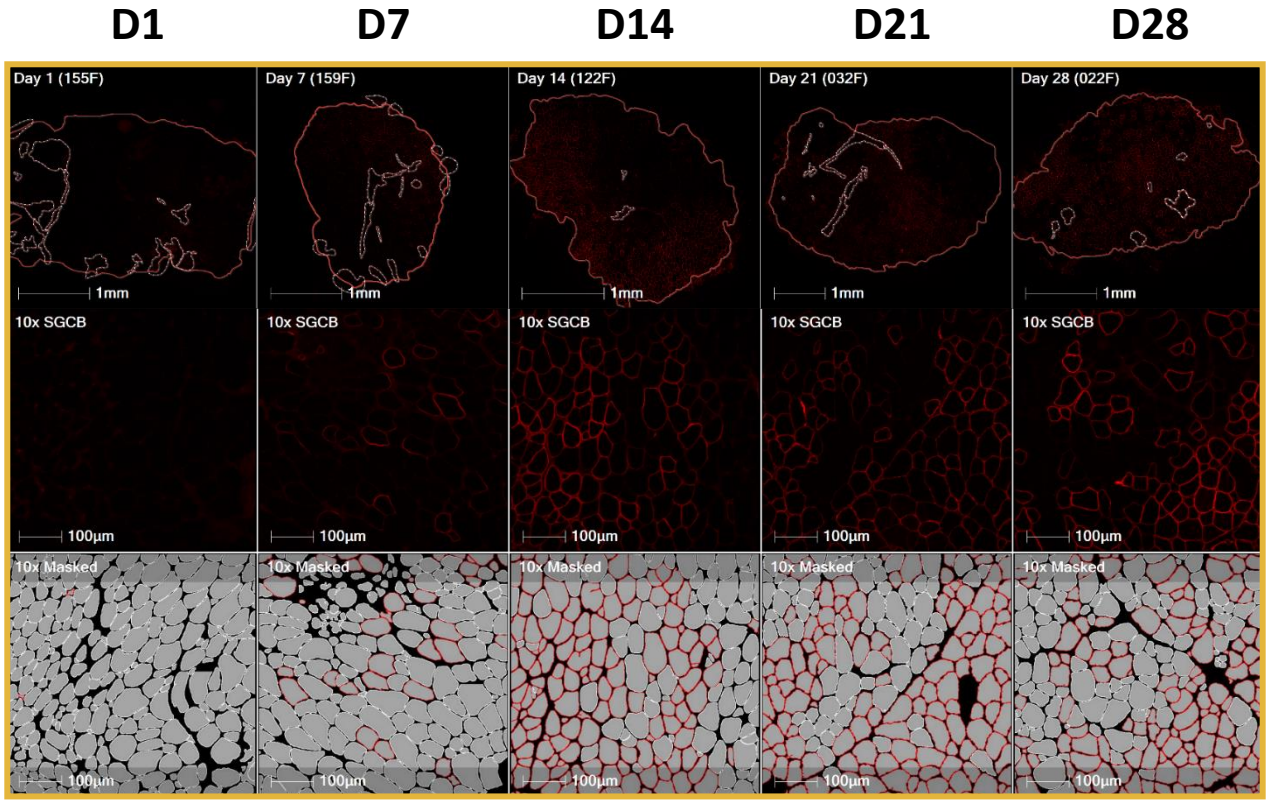


D=day; DAPI=4′,6-diamidino-2-phenylindole; FISH-IF=fluorescence in situ hybridization with immunofluorescence; IM=intramuscular; mRNA=messenger RNA; VG=vector genomes.

RESULTS
SGCB expression post SRP-9003 injection (IM)

- **SRP-9003 led to robust SGCB expression as early as D14 in skeletal muscle, with stabilization by D28**
- These data support **sustained SGCB protein expression** 28 days following IM injection

Immunofluorescent Staining of SGCB Protein Expression (PPF) Following IM Injection



D=day; IM=intramuscular; mRNA=messenger RNA; PPF, percent positive fibers; SGCB=β-sarcoglycan.

RESULTS

SRP-9003 – IV Administration

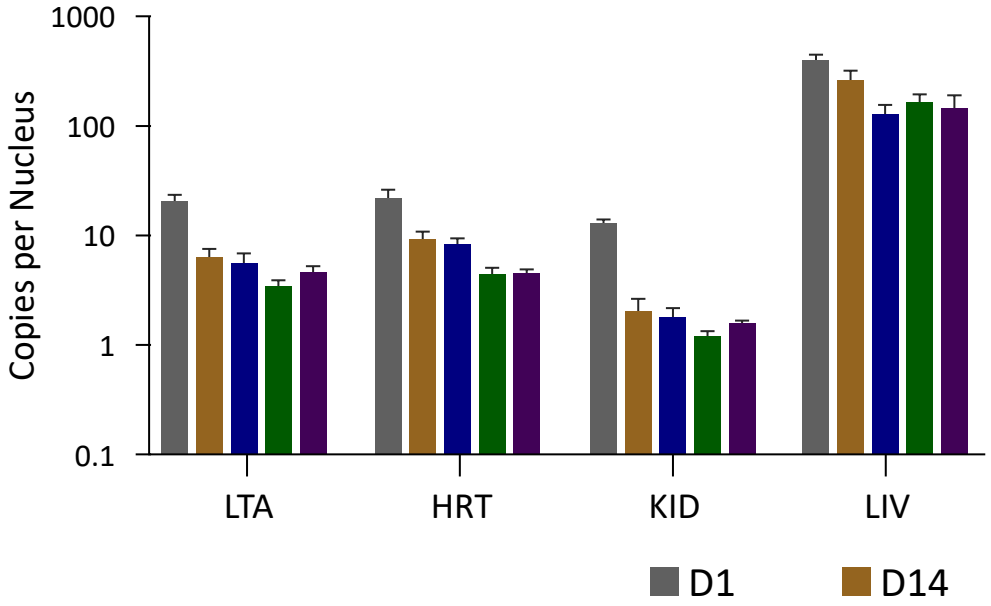
RESULTS

SRP-9003 biodistribution: transduction confirmation (IV)

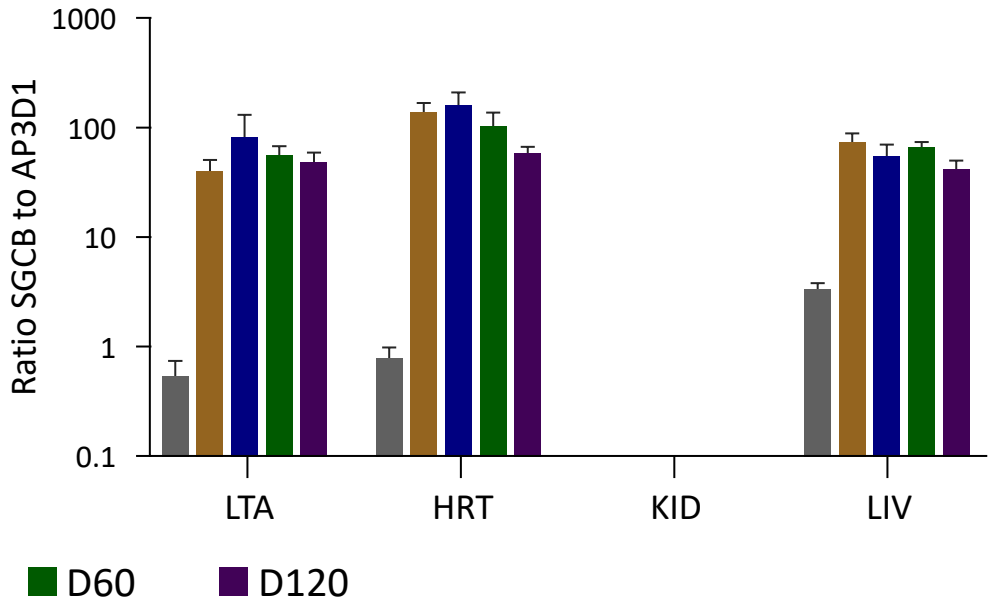
SRP-9003 DNA levels (copies/nucleus) **stabilized at D60 and were maintained through D120** following systemic administration

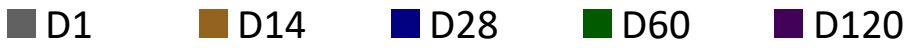
SRP-9003 hSGCB mRNA levels were **maximized by D14 and levels were equivalent by D120,** indicating vector durability following systemic administration

SRP-9003 Vector DNA Levels (ddPCR)



SRP-9003 hSGCB mRNA Levels (RT-ddPCR)





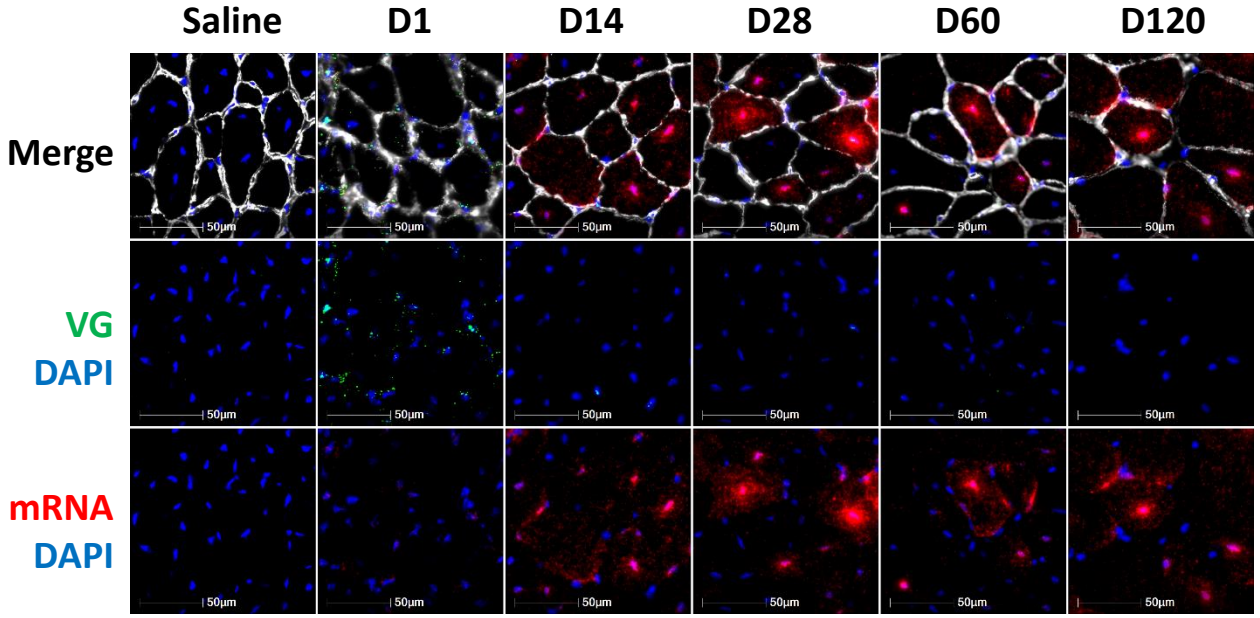
Note: error bars represent standard error of the mean.
AP3D1=adaptor-related protein complex 3 subunit delta 1; D=day; ddPCR=droplet digital polymerase chain reaction; HRT=heart; hSGCB=human β-sarcoglycan; IV=intravenous; KID=kidney; LIV=liver; LTA=left tibialis anterior; mRNA=messenger RNA; RT-ddPCR=reverse transcription droplet digital polymerase chain reaction; SGCB=β-sarcoglycan.

RESULTS

Detection of SRP-9003 in situ using RNAscope™ (IV): tibialis anterior

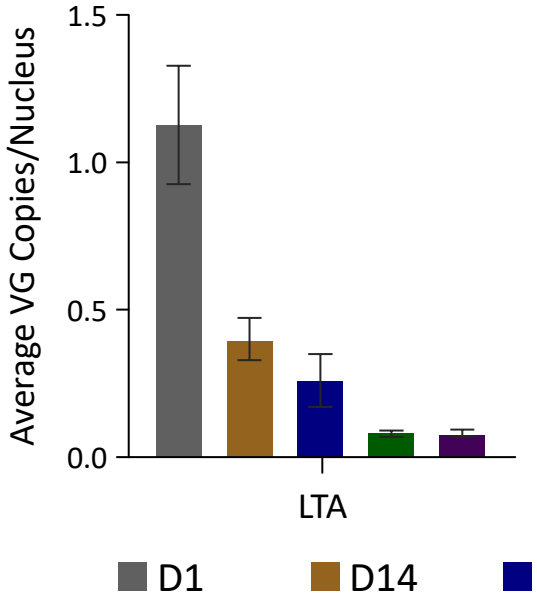
- In skeletal muscle, a gradual decline in vector DNA signal (RNAscope™) was detected from D1 to D120
- Despite low vector DNA levels, transgene mRNA signal was stable between D60 and D120

SRP-9003 Detection in Tibialis Anterior Using RNAscope™

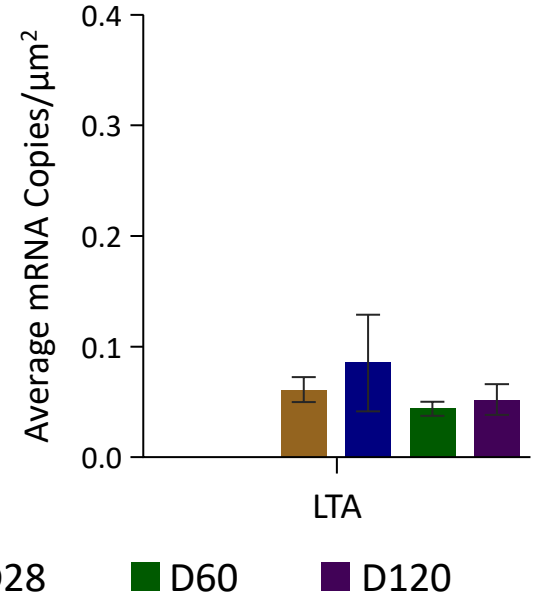


SRP-9003 dose: 7.41×10^{13} vg/kg

SRP-9003 Vector DNA Levels (RNAscope™)



SRP-9003 hSGCB mRNA Levels (RNAscope™)



D1 D14 D28 D60 D120

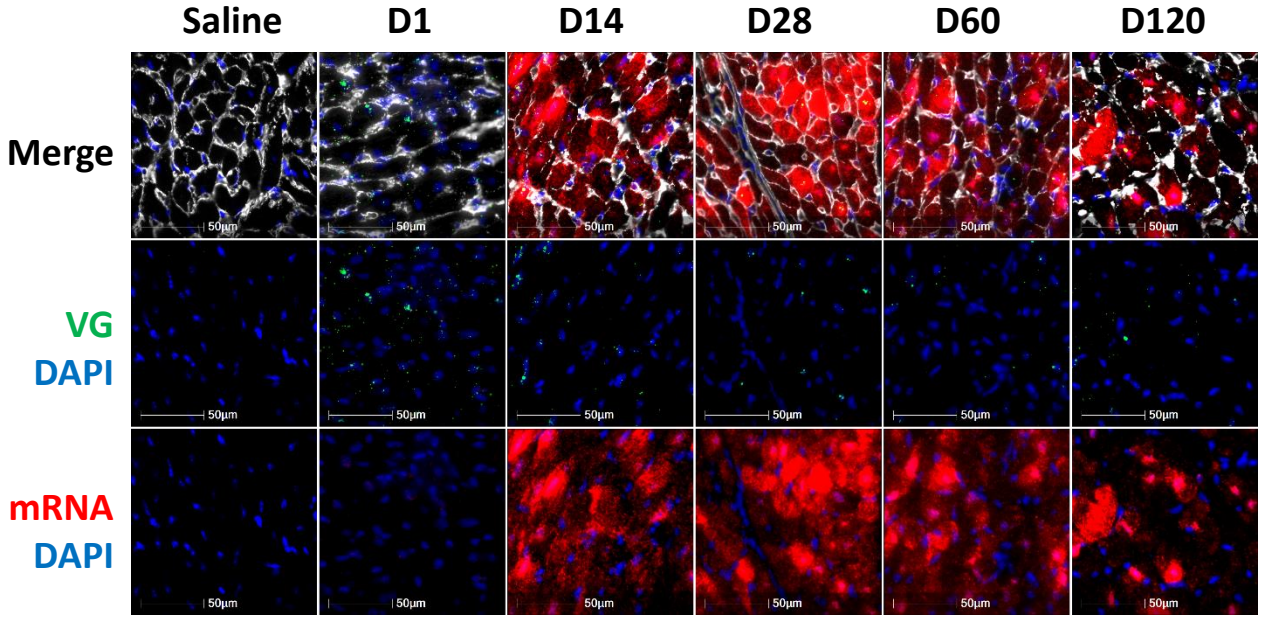
Note: error bars represent standard error of the mean.
D=day; DAPI=4',6-diamidino-2-phenylindole; hSGCB=human β-sarcoglycan; IV=intravenous; LTA=left tibialis anterior; mRNA=messenger RNA; VG=vector genomes.

RESULTS

Detection of SRP-9003 in situ using RNAscope™ (IV): heart

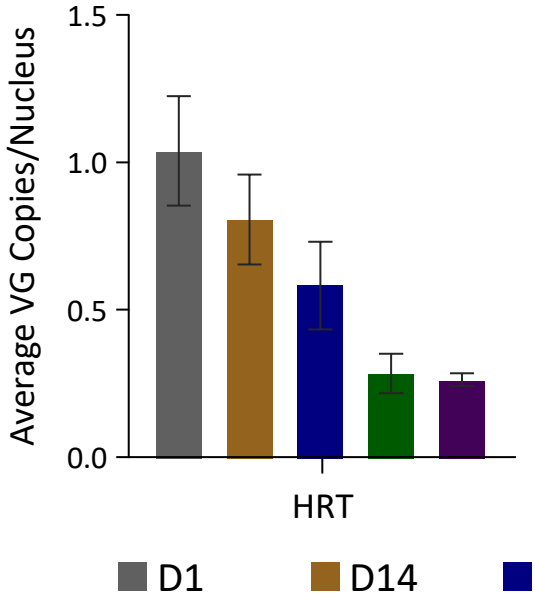
- In heart, vector DNA signal (RNAscope™) also gradually declined from D1 to D120
- Significant transgene mRNA signal was achieved in heart by D14 and was maintained 60–120 days post injection

SRP-9003 Detection in Heart Using RNAscope™

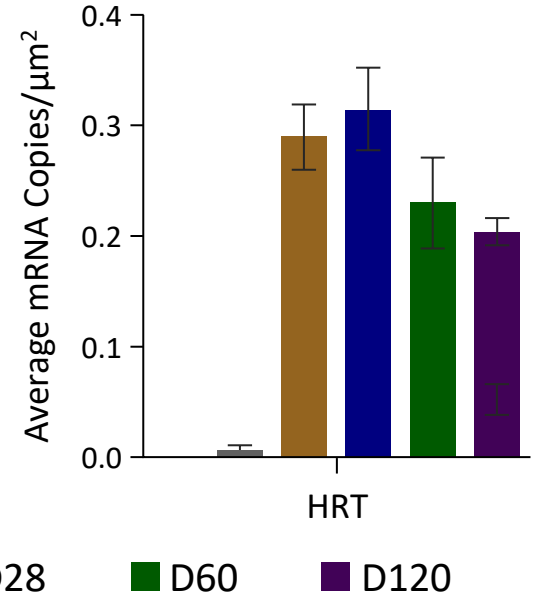


SRP-9003 dose: 7.41×10^{13} vg/kg

SRP-9003 Vector DNA Levels (RNAscope™)



SRP-9003 hSGCB mRNA Levels (RNAscope™)



Note: error bars represent standard error of the mean.

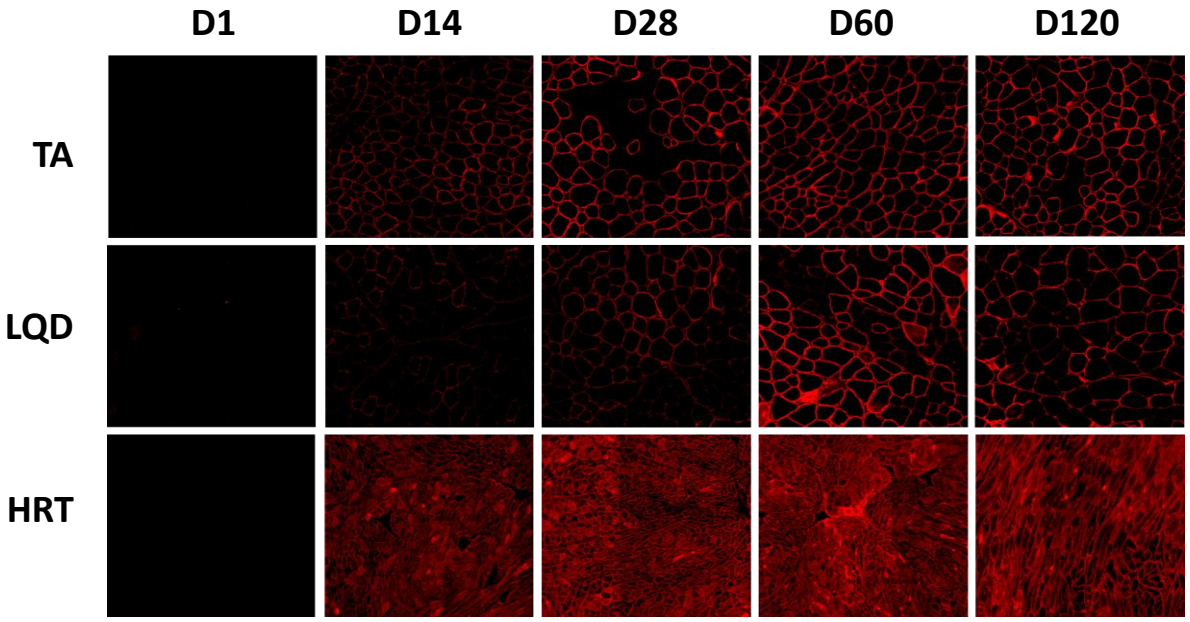
D=day; DAPI=4',6-diamidino-2-phenylindole; HRT=heart; hSGCB=human β-sarcoglycan; IV=intravenous; mRNA=messenger RNA; VG=vector genomes.

RESULTS

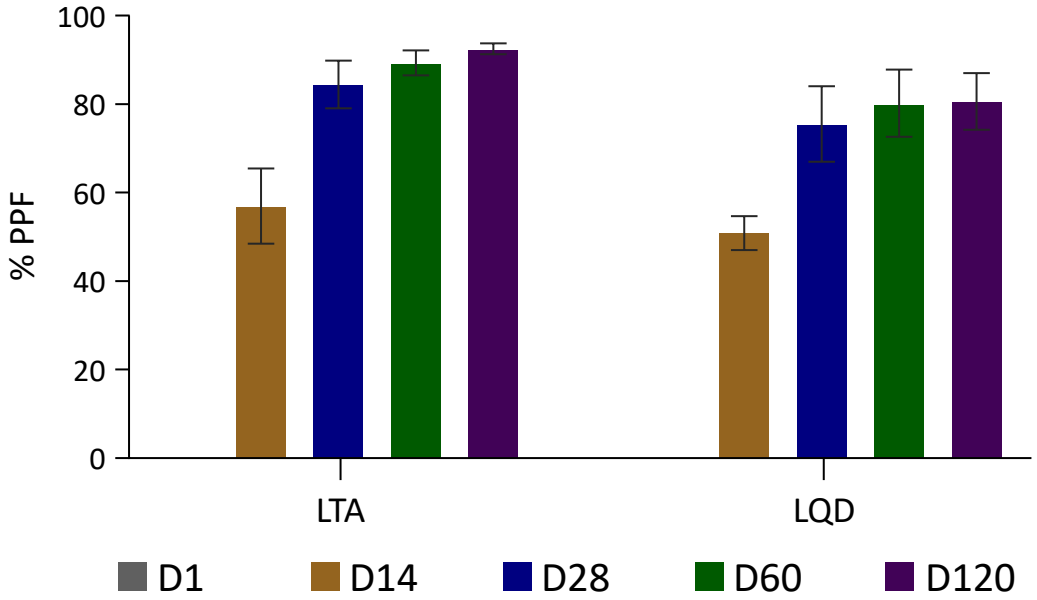
SRP-9003: durable SGCB expression 120 days post dose (IV)

- SGCB protein expression (PPF) remained robust up to D120
- Supports examination of protein expression at D60 for clinical trials (SRP-9003-101, SRP-9003-102, and SRP-9003-301) as SGCB expression is maximized at D60

SGCB Protein Expression (PPF) Following IV Injection



SGCB Expression (PPF)



Note: error bars represent standard error of the mean.
D=day; HRT=heart; IV=intravenous; LTA=left anterior tibialis; LQD=left quadricep; PPF=percent positive fibers; SGCB=β-sarcoglycan.

CONCLUSIONS
Key Findings

- **RNAscope™ determined the spatial, cellular organization, and molecular trafficking of SRP-9003**
- **Results indicate the durability of SRP-9003 to generate stable SGCB mRNA and protein expression over extended periods in a mouse model of LGMD2E/R4**
- **These data may inform future application of RNAscope™ for biodistribution evaluation of AAVrh74 gene therapy**

AAVrh74=adeno-associated virus serotype rh74; LGMD2E/R4=limb-girdle muscular dystrophy type 2E; mRNA=messenger RNA; SGCB=β-sarcoglycan.

DISCUSSION
Limitations

- **Limited/no ability to differentiate from dsDNA and episomal AAV DNA (ACD) using RNAscope™**
  - Lack of denaturing step (heat) to preserve tissue integrity
  - Expectation would likely be decreased vector genome copies over time (1 month vs 6 months) based on Zhao 2020 (AAV9)[1]
  - Future evaluations will examine heating step in similar tissues

- **The assay is unable to detect *integrated* AAV vector DNA**

AAV=adeno-associated virus; ACD=Advanced Cell Diagnostics, Inc.; dsDNA=double-stranded DNA.

1. Zhao J, et al. *Mol Ther Methods Clin Dev*. 2020;18:856-68.

THANK YOU

For questions please contact: medinfo@sarepta.com

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