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In Situ Biodistribution and Localization of Bidridistrogene Xeboparvovec (SRP-9003) in LGMD2E/R4 Mice After 1 Year of Follow-up

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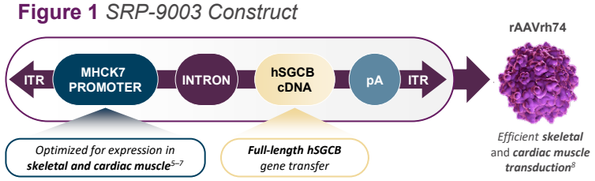
Introduction

- Limb-girdle muscular dystrophy type 2E/R4 (LGMD2E/R4) is caused by pathogenic variants in the β-sarcoglycan (*SGCB*) gene and results in a loss of functional SGCB protein, progressive muscle degeneration, and shortened lifespan[1,2]
- Bidridistrogene xeboparvovec (SRP-9003, rAAVrh74.MHCK7.hSGCB) is an investigational adeno-associated virus (AAV)-based gene therapy designed to restore functional SGCB expression in muscle and improve clinical outcomes in patients with LGMD2E/R4 (**Figure 1**)[3]
- The maintenance of SRP-9003 vector DNA and transgene mRNA up to 120 days following systemic administration in LGMD2E mice has been previously demonstrated[4]

Figure 1 *SRP-9003 Construct*



cDNA=complementary DNA; hSGCB=human β-sarcoglycan; ITR=inverted terminal repeat; MHCK7=myosin heavy-chain muscle creatine kinase promoter; pA=polyadenylation; rAAVrh74=recombinant adeno-associated virus serotype rh74.

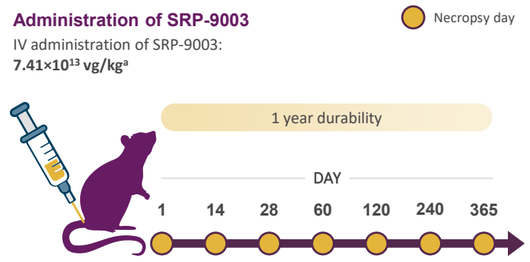
Objective

To evaluate the in situ spatial biodistribution, cellular tropism, transgene expression, and durability of SRP-9003 up to 1 year following intravenous (IV) administration in LGMD2E/R4 mice

Methods

- The administration of SRP-9003 and necropsy timepoints for the mouse model (n=5) are described in **Figure 2**
- Subcellular localization of vector genomes and mRNA were assessed using RNAscope™, which employs an in situ hybridization (ISH) technique to detect targeted nucleic acid levels at single molecule resolution within tissues and cells[9,10]
- The ISH probes used for RNAscope™ were designed to be non-overlapping for two-plex ISH
- Two-plex ISH allowed for the evaluation of subcellular localization of vector genome DNA and h*SGCB* RNA for spatial analysis
- Droplet digital polymerase chain reaction (ddPCR), reverse transcription-droplet digital polymerase chain reaction (RT-ddPCR), and immunofluorescence (IF) methods are described in the Supplemental Methods

Figure 2 *Study Design*



[a]Dose is calculated based on a linearized plasmid standard.[3]
IV=intravenous; vg=vector genomes.

Results

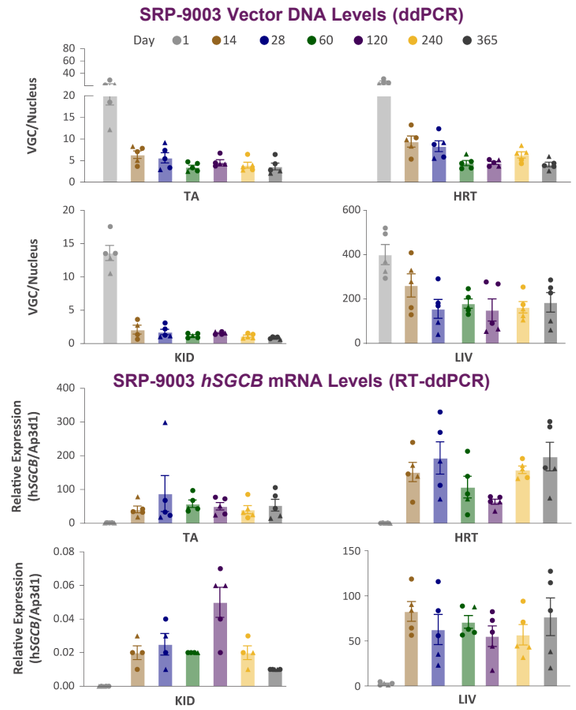
SRP-9003 Biodistribution

- SRP-9003 vector DNA levels (ddPCR) and mRNA expression (RT-ddPCR) were maintained through Day (D) 365 in skeletal and heart muscle, indicating vector durability over extended periods in LGMD2E/R4 mice (**Figure 3**)
- Considering preclinical evidence from 12-week pharmacology studies, SRP-9003 biodistribution at D365 was expected[11]
- No significant differences were seen between D60 and any subsequent timepoint in vector DNA and mRNA expression in all tissues shown here (**Figure 3**)
- In off-target organs such as the liver and kidney, detectable SRP-9003 vector DNA and RNA were also present up to D365, but minimal mRNA expression was detected in the kidney (**Figure 3**)

Detection of SRP-9003 In Situ and Durable SGCB Expression

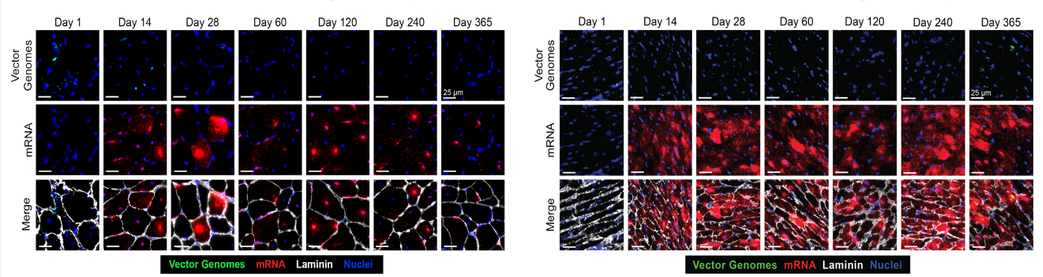
- In skeletal muscle, tibialis anterior (TA), vector DNA signal (RNAscope™) gradually declined from D1 to D240, but transgene mRNA signal was stable between D14 and D240 (**Figures 4, 5**)
- In the heart, vector DNA signal gradually declined from D1 to D120 but stabilized at D240 and D365 (**Figures 4, 5**)
- A durable transgene mRNA signal was achieved in the heart by D14 and was maintained to D365 post injection (**Figures 4, 5**)
- No RNAscope™ mRNA signal was detected in the kidney over the course of the study (data not shown)
- SGCB protein expression (percent positive fibers [PPF]) maximized at D240 and remained elevated up to D365 in TA (**Figure 6**)

Figure 3 *SRP-9003 Vector DNA and Transgene mRNA in Skeletal Muscle and Organs Following 365 Days of Treatment*



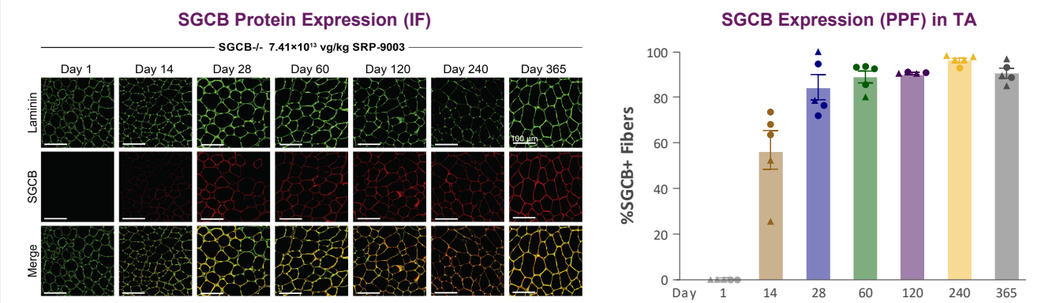
Representative histograms quantifying SRP-9003 vector DNA and transgene mRNA over duration of study. Note: Error bars represent standard error of the mean. Individual data points are represented as circles (male) and triangles (female). Ap3d1=adaptor-related protein complex 3 subunit delta 1; ddPCR=droplet digital polymerase chain reaction; HRT=heart; h*SGCB*=human β-sarcoglycan; KID=kidney; LIV=liver; RT-ddPCR=reverse transcription-ddPCR; TA=tibialis anterior; VGC=vector genome copies.

Figures 4, 5 *Detection of SRP-9003 In Situ Using RNAscope™: TA and HRT*



Representative RNAscope™ images of the tibialis anterior (TA) and heart (HRT) muscles from SGCB-/- mice treated with SRP-9003 showing continued h*SGCB* mRNA expression from 1 day to 365 days post treatment. Laminin (shown in white) represents the fiber membrane. Vector genomes are shown in green, and mRNA is shown in red. Scale bar = 25 µm.
h*SGCB*=human β-sarcoglycan; SEM=standard error of the mean.

Figure 6 *SGCB Membrane-Localized Protein Restoration Is Sustained*



Representative immunofluorescence (IF) images of tibialis anterior (TA) muscle in SGCB-/- mice treated with SRP-9003 show restoration of β-sarcoglycan (SGCB) to the cell membrane and sustained expression through 365 days post treatment. Laminin (green) represents the fiber membrane, SGCB (red) represents β-sarcoglycan expression, and the yellow merge represents localization of β-sarcoglycan to the fiber membrane.
Scale bar = 100 µm. Bars represent the mean ± SEM. No statistical significance testing was performed on the percent positive fiber (PPF) data.
SEM=standard error of the mean.

Conclusions

This study demonstrates the applicability of RNAscope™ to determine 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 of up to 1 year in a mouse model of LGMD2E/R4

These outcomes show that D60 represents an appropriate time frame to evaluate expression in clinical biopsies, and the data demonstrate a consistent expression through at least 1 year of treatment

These data may be useful to inform future application of RNAscope™ for biodistribution evaluation of AAVrh74 gene therapies

Presented at the American Society of Gene and Cell Therapy (ASGCT) Annual Meeting;

May 13–17, 2025; New Orleans, LA

Acknowledgments & Disclosures

This study was funded by Sarepta Therapeutics, Inc. Editorial support was provided by Kimberly Fischer, PhD, of Eloquent Scientific Solutions and was funded by Sarepta Therapeutics, Inc. **SB, YS, SF, JG, CH, AP, JW, AH, SL, LR-K, RP**: All authors are employees of Sarepta Therapeutics, Inc., and may own stock/options in the company.

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Methods (cont)

Supplemental Methods

- **Droplet digital polymerase chain reaction (ddPCR)**
  - DNA isolates collected were analyzed by ddPCR for presence of vector genomes using a vector-specific primer probe set designed to amplify a sequence in the myosin heavy-chain muscle creatine kinase promoter (MHCK7)
  - Beta-actin was used as reference gene to quantify the amount of genomic DNA
- **Reverse transcription-droplet digital polymerase chain reaction (RT-ddPCR)**
  - RNA isolates were collected and analyzed by RT-ddPCR using a transgene-specific primer probe set designed to amplify a sequence in the h*SGCB* transgene
  - A second set of primer and probes against adaptor-related protein complex 3 subunit delta 1 (Ap3d1) was used as reference
- **Immunofluorescence (IF)**
  - IF assay was performed to assess hSGCB protein expression
  - The percent of hSGCB-positive fibers was determined using HALO IA algorithms (Indica Labs, Albuquerque, NM) for each image for percent positive fibers (PPF) analysis
- **RNAscope™**
  - Visualization of subcellular localization of vector genomes and h*SGCB* RNA following IV injection of SRP-9003 occurred using two-plex ISH
  - One probe detected AAVrh74-vector DNA only (antisense strand) and a second probe captured h*SGCB* transgene mRNA (sense strand)
  - Quantitative analysis of vector genome and h*SGCB* RNA was performed using fluorescence in situ hybridization (FISH) and FISH-IF algorithm

Limitations

- Limited ability/not able to differentiate from double-stranded DNA and episomal AAV DNA (Advanced Cell Diagnostics, Inc.) using RNAscope™
- Lack of denaturing step (heat) to preserve tissue integrity. Future evaluations will examine heating step in similar tissues
- The assay is unable to detect integrated AAV-vector DNA

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