

Preclinical Evaluation of SRP-1003: An αvβ6 Integrin-Targeting siRNA for Nuclear-Targeted Knockdown of *DMPK* in Myotonic Dystrophy Type 1

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Background

- Myotonic dystrophy type 1 (DM1) is an inherited, progressive, multi-system, autosomal dominant muscular dystrophy^{1,2}
- DM1 is caused by a CTG trinucleotide repeat expansion within the 3'-UTR of the dystrophia myotonica protein kinase (*DMPK*) gene^{1,3}
- When expressed, CUG-repeat expanded *DMPK* messenger RNA (mRNA) forms large hairpins within the nucleus that sequester muscleblind-like (MBNL) splicing regulators⁴
- Nuclear sequestration of MBNL family splicing factors causes an adult-to-fetal isoform spliceopathy, leading to highly variable disease presentation and severity in multiple organ systems, including the central nervous system and striated muscle⁵

- In addition, CUG-repeat expanded RNA increases CUGBP1 (a splicing regulator also known as CELF1) abundance and activity, further contributing to mis-splicing and DM1 pathogenesis⁶
- SRP-1003 is an investigational small interfering RNA (siRNA) that degrades *DMPK* mRNA, reducing toxic CUG-repeat RNA foci and improving downstream splicing

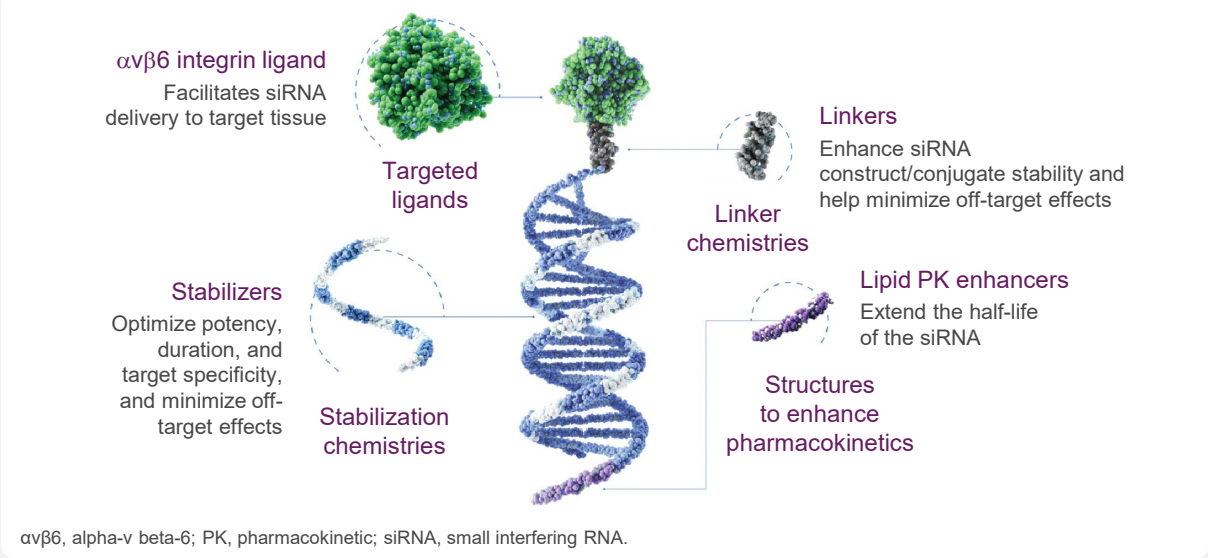
Objective

- To characterize the biodistribution of SRP-1003 in rats and demonstrate nuclear efficacy in DM1 preclinical models

Methods

- SRP-1003 utilizes an siRNA with unique chemical modifications with an αvβ6 integrin-targeting ligand and lipid moiety for muscle delivery and enhanced pharmacokinetic/pharmacodynamic properties (**Figure 1**)

Figure 1 SRP-1003, an αvβ6 Integrin-Targeting siRNA Therapy^{7,8}



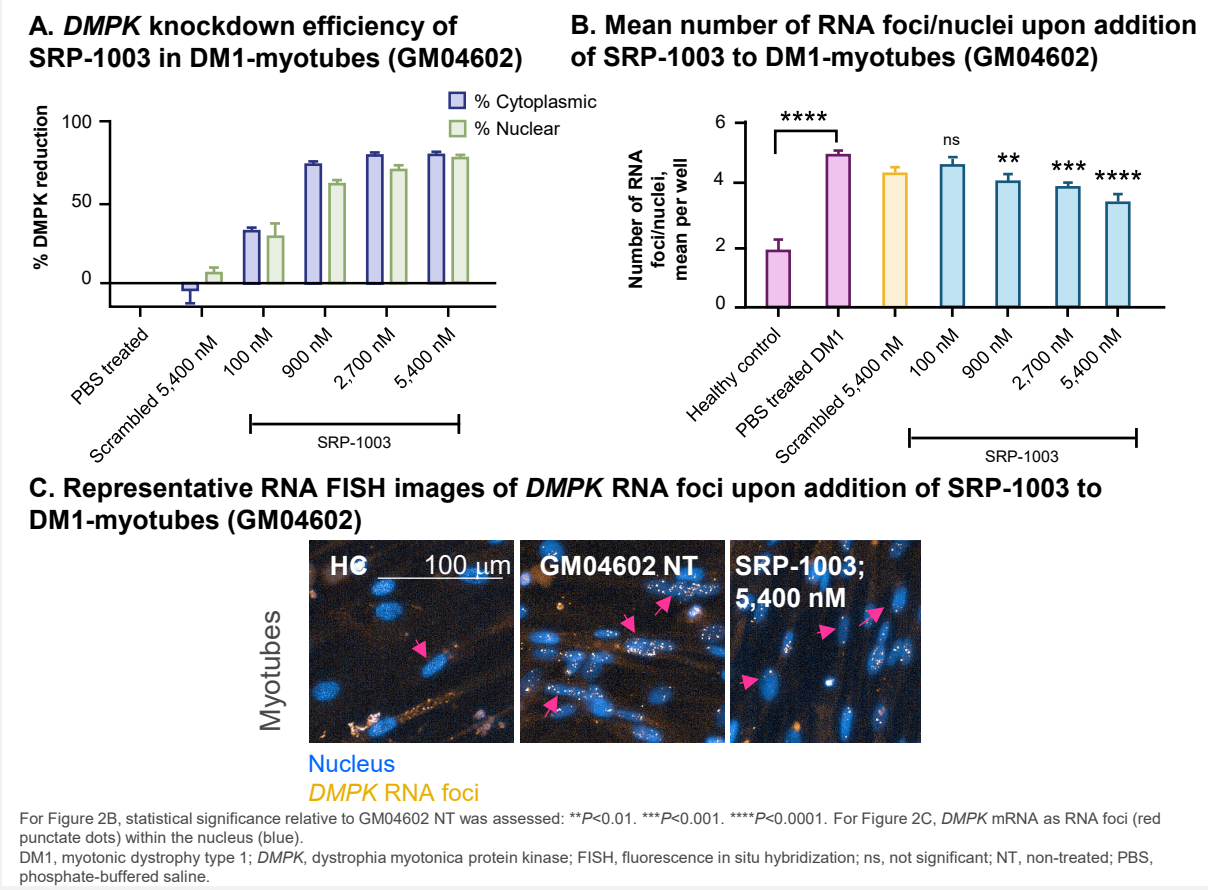
Animal/cell models

- To assess SRP-1003 pharmacokinetics, a single 8 mg/kg intravenous (IV) siRNA bolus dose was administered to male Sprague Dawley rats (n=4)
 - Blood samples were collected at various timepoints up to 16 weeks; plasma concentrations of SRP-1003 were quantified using a peptide nucleic acid hybridization method, which detects the antisense strand
- DM1 patient–derived, induced pluripotent stem cells with ~1,600 CTG repeats (GM04602) were differentiated into myotubes and used to assess SRP-1003–induced knockdown of cytoplasmic and nuclear *DMPK* mRNA and RNA foci
 - GM04602 myotubes and healthy human control myotubes were treated with phosphate-buffered saline to serve as controls
 - Four-day pre-differentiated GM04602 myotubes were incubated with SRP-1003 for 2 days; then, myotubes were maintained for an additional 5 days
 - Seven days following the addition of SRP-1003, cytoplasmic and nuclear RNA were fractionated using Norgen’s cytoplasmic and nuclear RNA purification kit, and *DMPK* mRNA expression was assessed by droplet digital polymerase chain reaction (ddPCR)
 - The purity of the cytoplasmic and nuclear RNA fractions was assessed for *HPRT* mRNA expression (cytoplasmic control) and *U6* (nuclear control); each experiment was performed in biological duplicate with technical duplicates
- DM1-like (TREDT960i/HSA-rTA) mice with a doxycycline-inducible human (h)*DMPK*-CUG transgene with 960 CTG repeats and noncarrier control (NCAR) mice were administered doxycycline by diet to induce h*DMPK*-CUG expression
 - Weekly IV administration of S-ARO-DM1, an SRP-1003 surrogate siRNA, at either 18 or 35 mg/kg (siRNA dose) decreased h*DMPK*-CUG expression in a dose-dependent manner as assessed by ddPCR
 - Spliceopathy was analyzed by RNA sequencing using mRNA from the gastrocnemius of DM1-like and NCAR mice. Composite percent spliced-in (PSI) was analyzed for a 35-gene panel (the 20 most responsive genes are shown here)
- The DMSXL mouse model of DM1 with >1,000 CTG repeats was used to assess SRP-1003–induced knockdown of h*DMPK* mRNA by ddPCR and reduction of nuclear RNA foci by RNAscope™
 - DMSXL mice, aged 7 to 10 weeks, received 5 once-weekly doses of either saline or 12 mg/kg SRP-1003 via tail vein injection
 - For RNA foci quantification, formalin-fixed, fresh frozen tissues were sectioned and stained with a Cy3-(CAG)₇ probe

Results

- Following a single 8 mg/kg IV siRNA dose, SRP-1003 was detected in multiple lower and upper body muscles of Sprague Dawley rats at 16 weeks (**Supplementary Figure 1 via QR code**)
- Compartment-fractionated analysis, validated by cytoplasmic and nuclear RNA controls, demonstrated that SRP-1003 reduced nuclear *DMPK* mRNA to a degree comparable to cytoplasmic *DMPK* mRNA across the concentration range evaluated (**Figure 2**)
 - SRP-1003 significantly reduces *DMPK* mRNA in both the cytoplasm and the nucleus in a dose-dependent manner with a maximal knockdown of 80% in both compartments (**Figure 2A**)
 - DMPK* knockdown was specific to SRP-1003 and was not seen with a scrambled siRNA control (DR3049-1; 5,400 nM; **Figure 2B**)

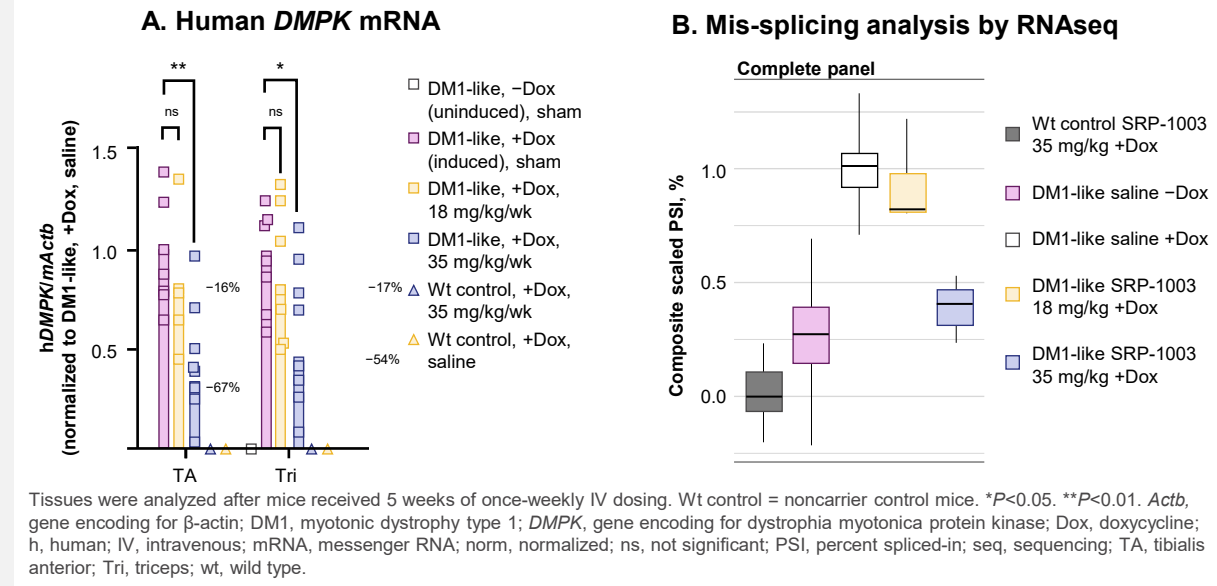
Figure 2 SRP-1003 Demonstrates Nuclear Efficacy in DM1-Myotubes (GM04602)



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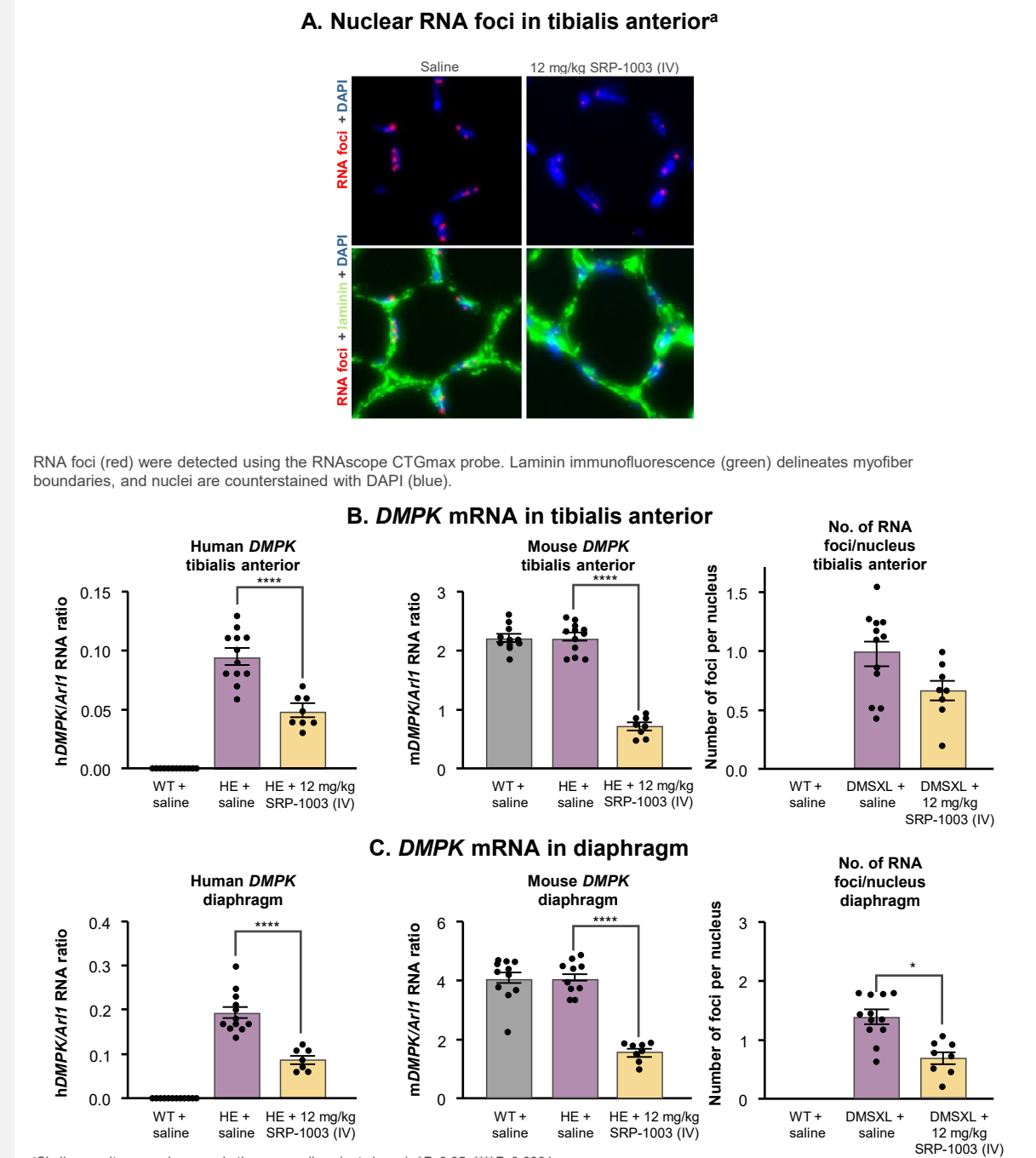
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Figure 3 SRP-1003 Reduces Expression of Human *DMPK* (A) and Exhibits Dose-Dependent Reversal of Splicing Deficits (B)⁹



- In the DMSXL mouse model, SRP-1003 (12 mg/kg IV) reduced nuclear RNA foci by RNAscope evaluation in tibialis anterior, diaphragm, and myocardium relative to saline-treated DMSXL controls (**Figure 4**) and resulted in significant knockdown of human *DMPK* and mouse *DMPK* mRNA (mean, ~60%) by reverse-transcription ddPCR (**Figure 4B and 4C**)

Figure 4 SRP-1003 Resulted in Reduction of Nuclear RNA in the DMSXL Mouse Model of DM1^a and Significant Knockdown of Human and Mouse *DMPK* mRNA in Tibialis Anterior and Diaphragm



^aSimilar results were also seen in the myocardium (not shown). *P<0.05. ****P<0.0001. Ar11, gene encoding for ADP-ribosylation factor-like 1; DAPI, 4',6-diamidino-2-phenylindole; DM1, myotonic dystrophy type 1; *DMPK*, gene encoding for dystrophia myotonica protein kinase; h, human; HE, hemizygous; IV, intravenous; m, mouse; mRNA, messenger RNA; WT, wild type.

Conclusions

- The αvβ6 integrin-targeting ligand and lipid moiety enabled systemic delivery of SRP-1003 to skeletal and cardiac muscle in rats and DM1 mice, with muscle exposure sustained for 16 weeks after a single dose
- SRP-1003 reduced nuclear-retained, CUG-expanded *DMPK* RNA in DM1 patient–derived myotubes and in the DMSXL mouse model, demonstrated by 3 independent methods: RNA fluorescence in situ hybridization (FISH) foci quantification, compartment-fractionated ddPCR with purity controls, and in situ RNAscope in intact cells
- Reduction of h*DMPK* mRNA in DM1-like mice was accompanied by correction of MBNL-dependent mis-splicing within skeletal muscle. Reduction of h*DMPK* in DMSXL mice was associated with reduced RNA foci in limb skeletal muscles, diaphragm, and myocardium
- Correction of MBNL-dependent splicing requires degradation of nuclear RNA transcripts, as both MBNL sequestration and pre-mRNA splicing occur exclusively within the nucleus
 - Cytoplasmic degradation of *DMPK* mRNA cannot release MBNL from nuclear foci; RNA foci reduction and splicing correction therefore constitutes functional evidence of siRNA-mediated target engagement within the nucleus, consistent with the established catalytic activity of RNA-induced silencing complex in the mammalian nuclear compartment
- These findings demonstrate SRP-1003 knocks down CUG-repeat expanded *DMPK* mRNA within the nucleus of DM1 preclinical models, leading to correction of MBNL-dependent splicing. This robust data set supports the continued clinical development of SRP-1003 in the ongoing DM1 phase 1/2 study (SRP-1003-101)

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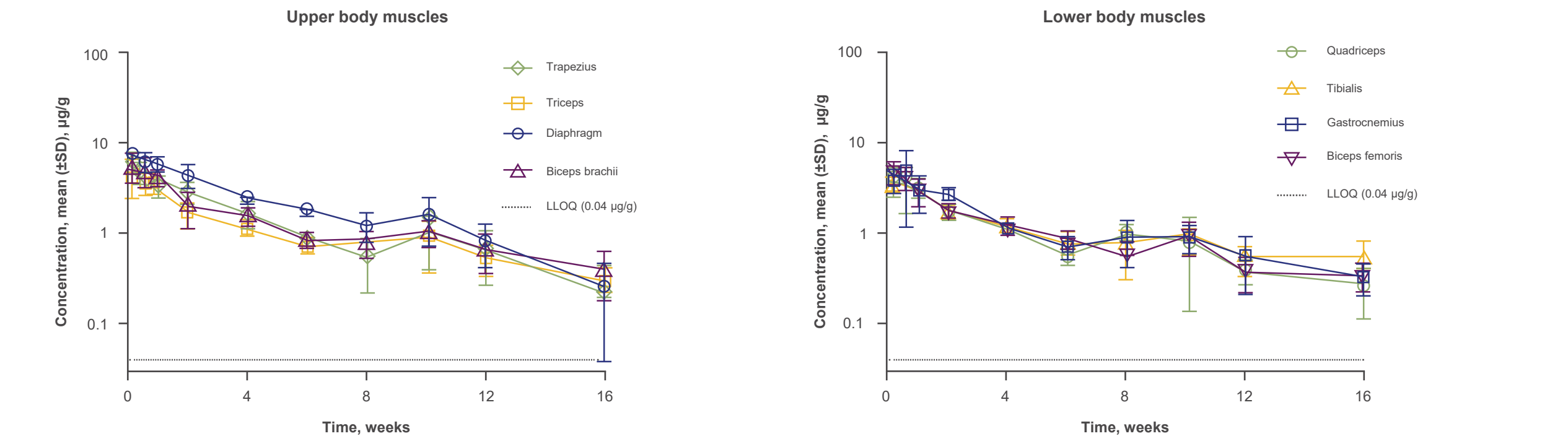
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Supplementary Results

- Following a single 8 mg/kg IV siRNA dose, SRP-1003 was detected in multiple upper and lower body muscles of Sprague Dawley rats at 16 weeks (**Supplementary Figure 1**)

Supplementary Figure 1 Tissue Concentrations in Rats Following a Single IV Bolus Dose of 8 mg/kg SRP-1003



IV, intravenous; LLOQ, lower limit of quantification; SD, standard deviation.

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