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Biological Efficacy of the Peptide-Conjugated Phosphorodiamidate Morpholino Oligomer SRP-5051 in Preclinical Models of Duchenne Muscular Dystrophy

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Objective

To investigate the biological efficacy of the peptide-conjugated phosphorodiamidate morpholino oligomer (PPMO) SRP-5051, designed to skip exon 51 of the *DMD* gene, in preclinical models

Key Takeaways

- **Dose-dependent efficacy of SRP-5051 was demonstrated in myotubes, mice, and nonhuman primate preclinical models**
  - **These data justify the SRP-5051 dosing regimen (every 4 weeks) used in ongoing clinical studies and support further clinical investigation**

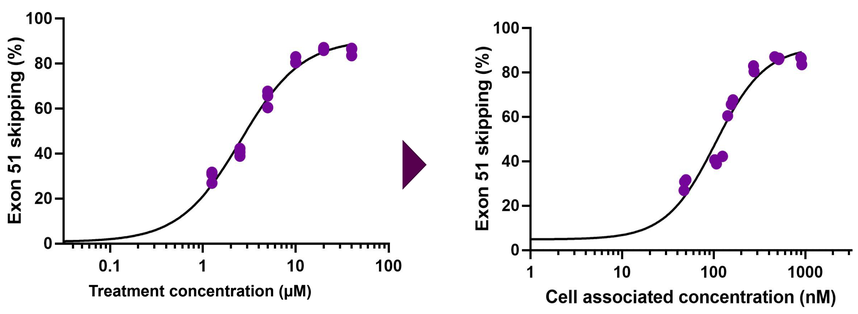
CONCLUSIONS

- Exon skipping and dystrophin production were demonstrated in Duchenne muscular dystrophy (DMD) patient myotubes *in vitro* at cellular SRP-5051 concentrations of 100−1000 nM
- In h*DMD*del52/*mdx* mice, a single SRP-5051 injection resulted in dose-dependent increases in exon skipping and human dystrophin protein
  - Repeated dosing every 4 weeks improved pharmacodynamic effects, with dystrophin protein accumulation through 20 weeks (5 doses) and sustained grip strength improvement through 20 weeks
- In nonhuman primates, a single SRP-5051 infusion resulted in a dose-dependent increase in exon skipping and accumulation of drug in muscle for up to 28 days
  - Repeated dosing every 4 weeks over 12 weeks showed exon skipping increased with each infusion, and SRP-5051 appeared to be well tolerated
- SRP-5051 showed dose-dependent efficacy in preclinical models, justifying the SRP-5051 dosing regimen (every 4 weeks) used in ongoing clinical studies and supporting further clinical investigation of this PPMO

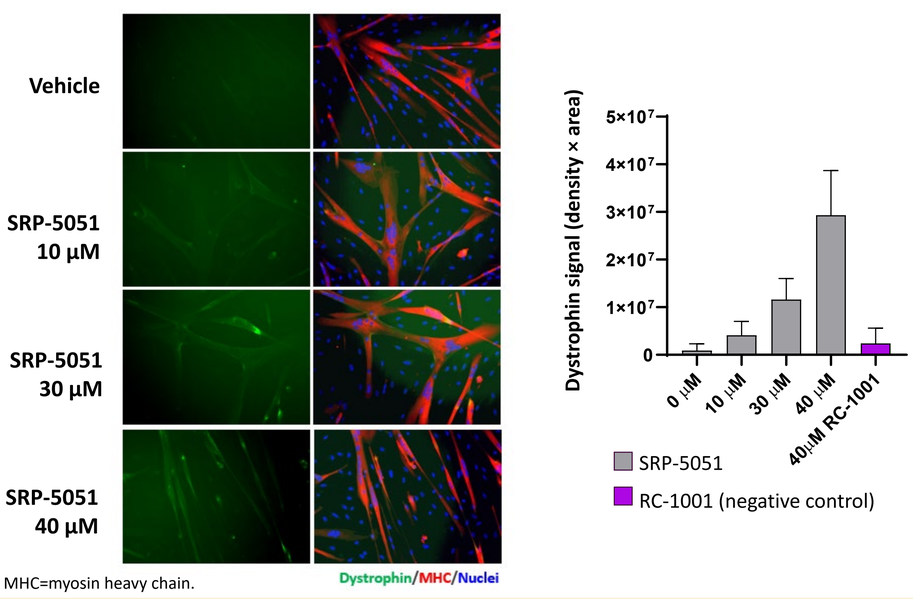
RESULTS

SRP-5051 dosing in a DMD patient-derived myotube model

SRP-5051 treatment dose-dependently drives exon skipping in DMD patient-derived myotubes at cellular concentrations of 100–1000 nM

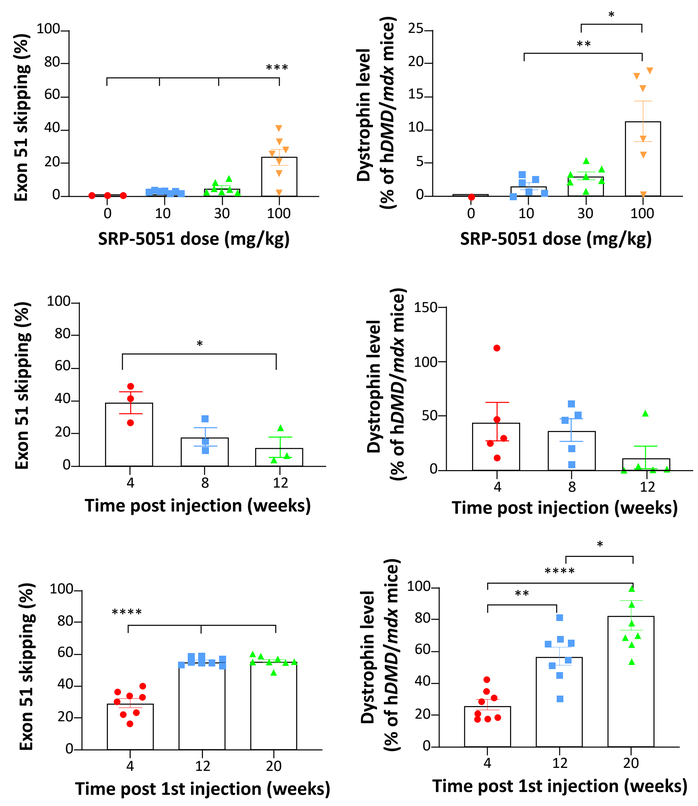


Concentration-dependent dystrophin production in *DMD* del52 myotubes was observed at SRP-5051 >10 μM



MHC=myosin heavy chain.

SRP-5051 dosing in h*DMD*del52/*mdx* mice



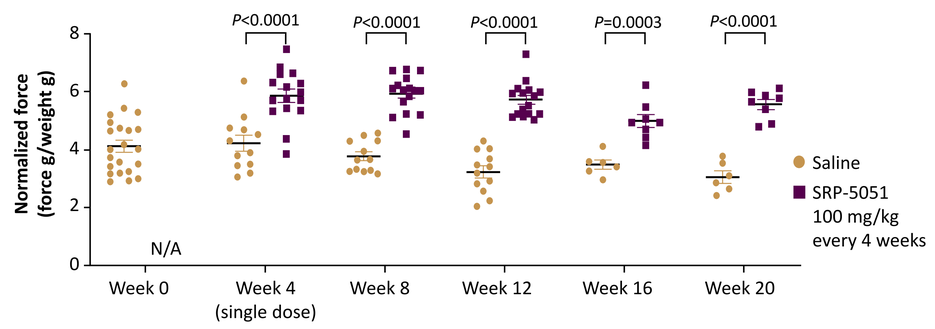
Exon skipping and dystrophin protein increased after a single dose of SRP-5051

Exon skipping and dystrophin protein levels were sustained 4 weeks after single-dose SRP-5051 and remained detectable to 12 weeks

SRP-5051 dosing every 4 weeks resulted in increased exon skipping and accumulation of dystrophin

Results for quadriceps are shown. Each bar represents mean ± SE (single dose and repeated dosing) or ± SD (single-dose time course). 1-way analysis of variance was used to compare means among dose groups. Dunnett multiple comparison test was used to compare the 2 groups of interest: **P*<0.05, ***P*<0.005, ****P*<0.0005, *****P*<0.0001.

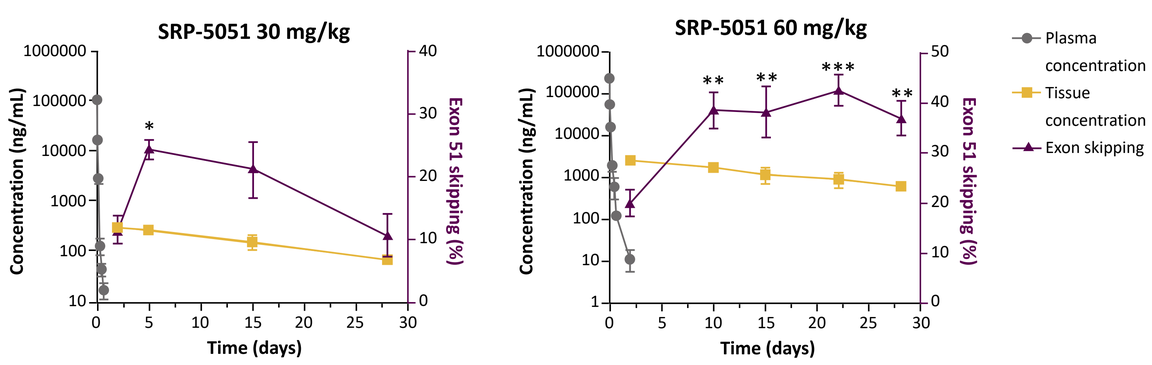
SRP-5051 restored grip strength and improvement was sustained throughout the 20-week study



Grip strength is a surrogate measure of muscle function. Unpaired *T*-test was used to compare the grip strength between the 2 groups at each time point.

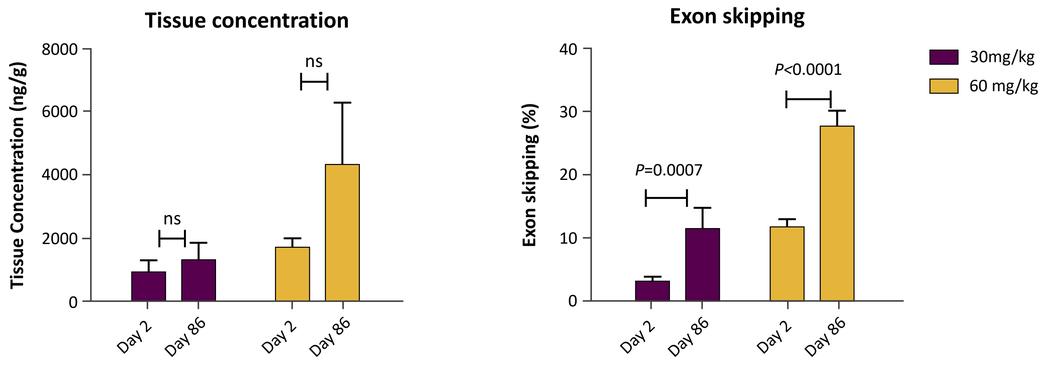
SRP-5051 treatment in nondystrophic nonhuman primates

Single dose of SRP-5051 results in accumulation in muscle tissue and exon 51 skipping that last for ≥28 days



**P*<0.05 (day 2). ***P*<0.01 (day 2). ****P*<0.001 (day 2).
Error bars represent SD. 2-way analysis of variance followed by Sidak's multiple comparison test used to compare exon skipping at different sampling times to earliest time point. Concentration determined in tissue by hybridized enzyme-linked immunosorbent assay (HELISA)

Repeated dosing every 4 weeks over 12 weeks showed dose-dependent tissue exposure and exon 51 skipping, and no new safety signals were detected



ns=not significant. Error bars represent SD.

ACKNOWLEDGMENTS & DISCLOSURES

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BACKGROUND

- Duchenne muscular dystrophy (DMD) is a severe, X-linked neuromuscular disease caused by mutations in the dystrophin (*DMD*) gene[1]
  - —Dystrophin mutations leading to deletions flanking exon 51 account for 13% of all patients with Duchenne muscular dystrophy (DMD)[2]
- Phosphorodiamidate morpholino oligomers (PMOs) are an effective treatment approach for patients with DMD[3-6]
- PMOs are designed for targeted skipping of exons within the *DMD* gene; they restore the reading frame and allow for production of an internally truncated but functional dystrophin protein
- Peptide-conjugated PMOs (PPMOs) are a next-generation chemistry platform in which a cell-penetrating peptide is conjugated to the PMO backbone, with the goal of increasing cellular uptake, exon skipping, and dystrophin production[7,8]
- SRP-5051 is an investigational PPMO designed to skip exon 51 of the *DMD* gene

METHODS DETAILS

Patient-derived myotube model

- Immortalized myoblasts derived from the paravertebral muscles of a healthy male donor and a male patient with DMD with an exon 52 deletion (*DMD* del52)
  - — Human telomerase and cyclin-dependent kinase 4 (CDK4)-immortalized myoblasts were provided to Sarepta by the Association Institut de Myologie (Paris, France); both donors were aged 16 years
- For DMD del52 cellular assays, myoblasts were allowed to differentiate for 2 days before treatment with PPMO (SRP-5051 or RC-1001) for 4 days
  - — RC-1001: negative control PPMO, with no homology to the human *DMD* gene
  - — Exon skipping was measured by droplet digital PCR (ddPCR)
  - — Dystrophin protein production was measured through imaging analysis
- To directly measure SRP-5051 cellular concentration by liquid chromatography–mass spectrometry, myoblasts were first allowed to differentiate for 3 days before treatment with PPMO (SRP-5051 or RC-1001) for 3 days, and washed
  - — Exon skipping was measured by ddPCR
  - — Dystrophin protein was measured by capillary Western

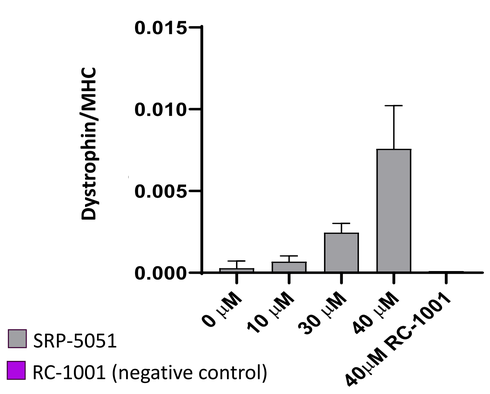
h*DMD*del52/*mdx* mouse model

- Human *DMD* transgene with exon 52 deleted by gene editing was inserted into the *mdx* mouse genome, resulting in an out-of-frame human *DMD* transcript and lack of dystrophin protein expression
- 6–8-week-old male h*DMD*del52/*mdx* mice were used for experiments
- Single dose
  - — Dose response: SRP-5051 IV at 10, 30, or 100 mg/kg; analyzed 7 days post dose for exon skipping and dystrophin
  - — Time course: SRP-5051 IV at 100 mg/kg; analyzed 4, 8, and 12 weeks post-dose for exon skipping and dystrophin
- Repeated doses
  - — SRP-5051 IV at 100 mg/kg every 4 weeks
    - Analyzed after 1, 3, or 5 doses for exon skipping and dystrophin
    - Analyzed every 4 weeks for grip strength

Nondystrophic nonhuman primate model

- Healthy male cynomolgus monkeys received a single 1-hour IV infusion of SRP-5051 at dose levels of 30 and 60 mg/kg
  - — Blood samples were collected on Day 1: predose and 1, 2, 4, 8, 12, 16, and 24 hours post infusion
  - — Muscle samples were collected on Days 2, 5 (only for the 30-mg/kg group), 10, 15, 22, and 28 by biopsy
- For repeated dosing, animals received 1-hour IV infusion of vehicle or SRP-5051 30 mg/kg or 60 mg/kg once every 4 weeks
  - — Muscle biopsy tissue was collected on the second day after each infusion

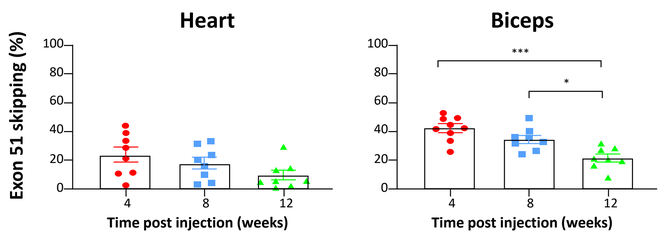
SUPPLEMENTARY RESULTS



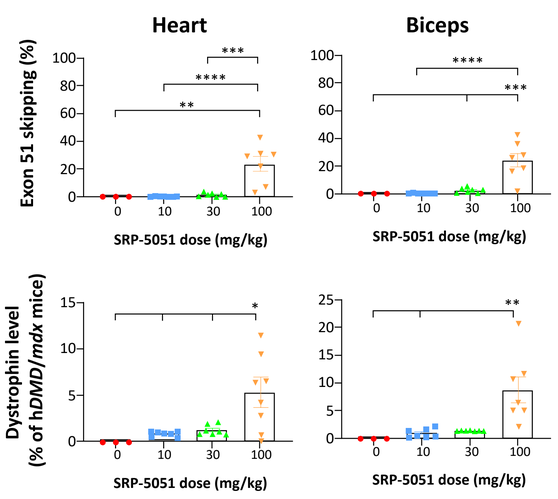
MHC=myosin heavy chain

Dystrophin protein production with SRP-5051 dosing in *DMD* del52 myotubes

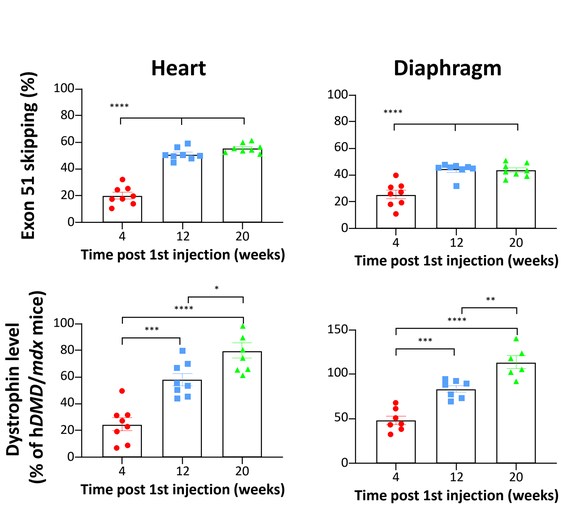
Exon skipping was sustained in heart and biceps 4 weeks after single-dose SRP-5051 and remained detectable to 12 weeks in h*DMD*del52/*mdx* mice



Each bar represents mean ± SD. 1-way analysis of variance was used to compare group means across 3 time points. Dunnett multiple comparison test was used to compare the 2 time points of interest: *P<0.05, ***P<0.005.



Single-dose SRP-5051 increased exon skipping and dystrophin protein in heart and biceps of h*DMD*del52/*mdx* mice



SRP-5051 dosing every 4 weeks resulted in increased exon skipping and accumulation of dystrophin in heart and diaphragm of h*DMD*del52/*mdx* mice

Each bar represents mean ± SE. 1-way analysis of variance was used to compare means among dose groups; Dunnett multiple comparison test was used to compare the 2 groups of interest: *P<0.05, **P<0.005, ***P<0.0005, ****P<0.0001.

Each bar represents mean ± SE. 1-way analysis of variance was used to compare group means. Dunnett multiple comparison test was used to compare the 2 groups of interest: *P<0.05, **P<0.005, ***P<0.0005, ****P<0.0001.

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