Development of Quantitative *In Vitro* Patient-Derived Cellular Models of Duchenne Muscular Dystrophy to Evaluate Peptide Phosphorodiamidate Morpholino Oligomers

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Disclosures

- Amy Erickson, Mark Wysk, Miralem Prijic, Leslie C.L. Wu, Jianbo Zhang, Annika B. Malmberg, Shawn Harriman, John R. Hadcock, and Peter M. Burch are employees of Sarepta Therapeutics, Inc. and may own stock in the company
- Huining Li, Andreea-Ilinca Visanoiu, Blandine Mille-Baker, and Fabian Stavenuiter are employees of Charles River Laboratories and may own stock in the company
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- Products are investigational only

Introduction

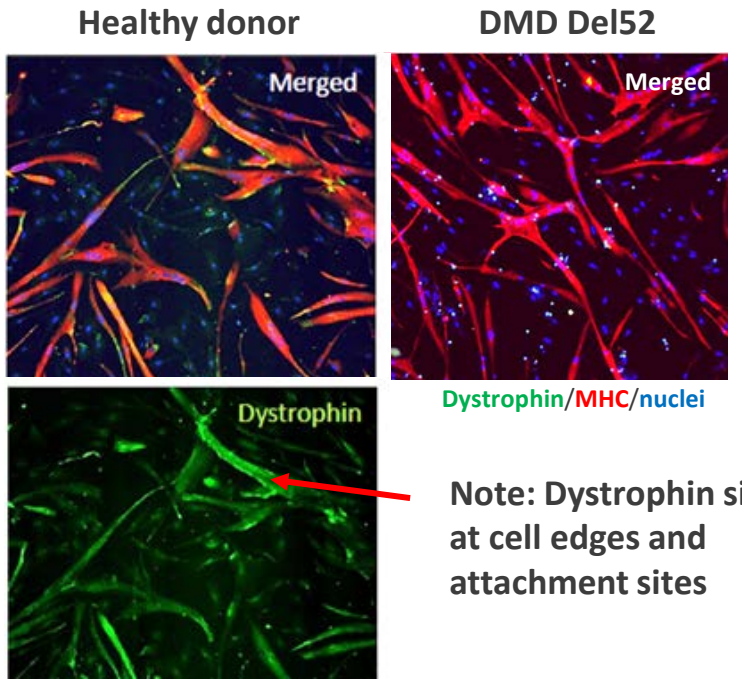
- Duchenne muscular dystrophy (DMD) is a severe, X-linked neuromuscular disease caused by mutations in the dystrophin gene[1]
  - Dystrophin mutations leading to deletions flanking exon 51 account for 13% of all DMD patients[2]
- Phosphorodiamidate morpholino oligomers (PMOs) are an effective treatment 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 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
- The development of PPMOs, like other potential therapies for DMD, has been hampered by a lack of disease-relevant quantitative *in vitro* pharmacology assays

Objective: To develop robust cell-based assays to quantitatively measure exon skipping and dystrophin production using gymnotic delivery of PPMOs

1. Birnkrant DJ, et al. *Lancet Neurol*. 2018;17:251-67. 2. Aartsma-Rus A, et al. *Hum Mutat*. 2009;30:293-9. 3. Popplewell LJ, et al. *Mol Ther.* 2009;17:554-61. 4. Exondys 51 [package insert]. Cambridge, MA: Sarepta Therapeutics, Inc.; 2020. 5. Vyondys 53 [package insert]. Cambridge, MA: Sarepta Therapeutics, Inc.; 2020. 6. Viltepso [package insert]. Paramus, NJ: NS Pharma, Inc.; 2020. 7. Gan L, et al. Poster presented at the 2019 Muscular Dystrophy Association (MDA) conference. April 13–17, 2019. Orlando, FL. 8. Echevarría L, et al. Hum Mol Genet. 2018; 27:R163-72.

DMD Patient-Derived Cell Model for Testing PPMOs

Immortalized myoblasts derived from the paravertebral muscles of a healthy male donor and a male DMD patient with an exon 52 deletion (DMD Del52)[a]



- Both lines grow well and differentiate into myotubes (**Myosin heavy chain** staining)
- Robust **dystrophin** staining in the healthy, but absent in DMD-patient myotubes with a signal-to-background ratio >10

Characterized a DMD patient-derived cell model with an exon 51 skipping-amenable mutation to test PPMOs

[a]Human telomerase and CDK4 immortalized myoblasts provided to Sarepta by the Association Institut de Myologie (Paris, France). Both donors were age 16 years.

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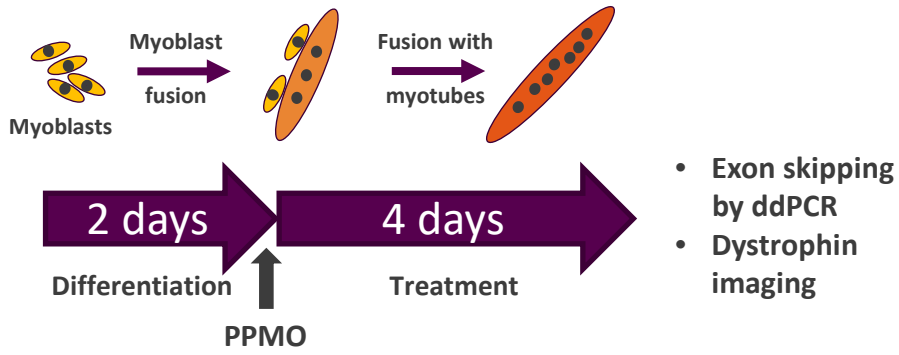
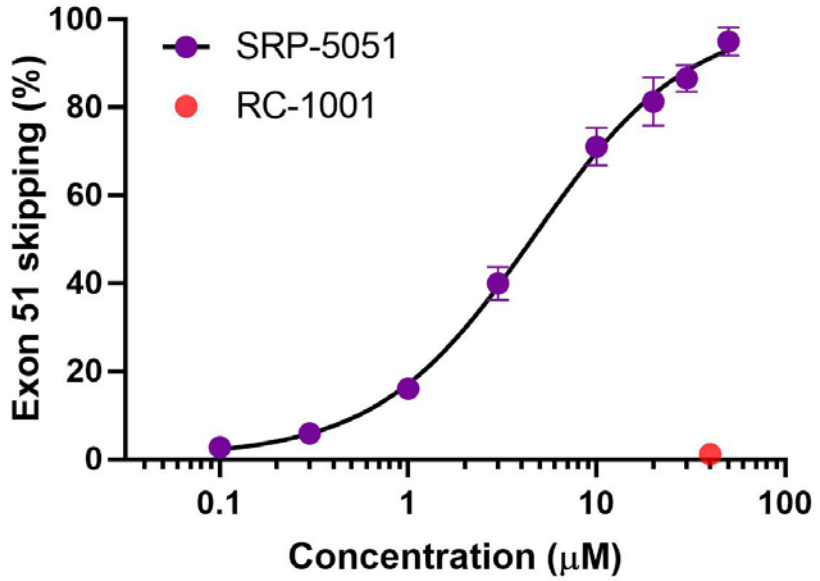
DMD Del52 Cellular Assay: Exon Skipping With SRP-5051



PPMO

- SRP-5051 (exon 51 skipping sequence)
- RC-1001 (negative control; no homology to the human *DMD* gene)

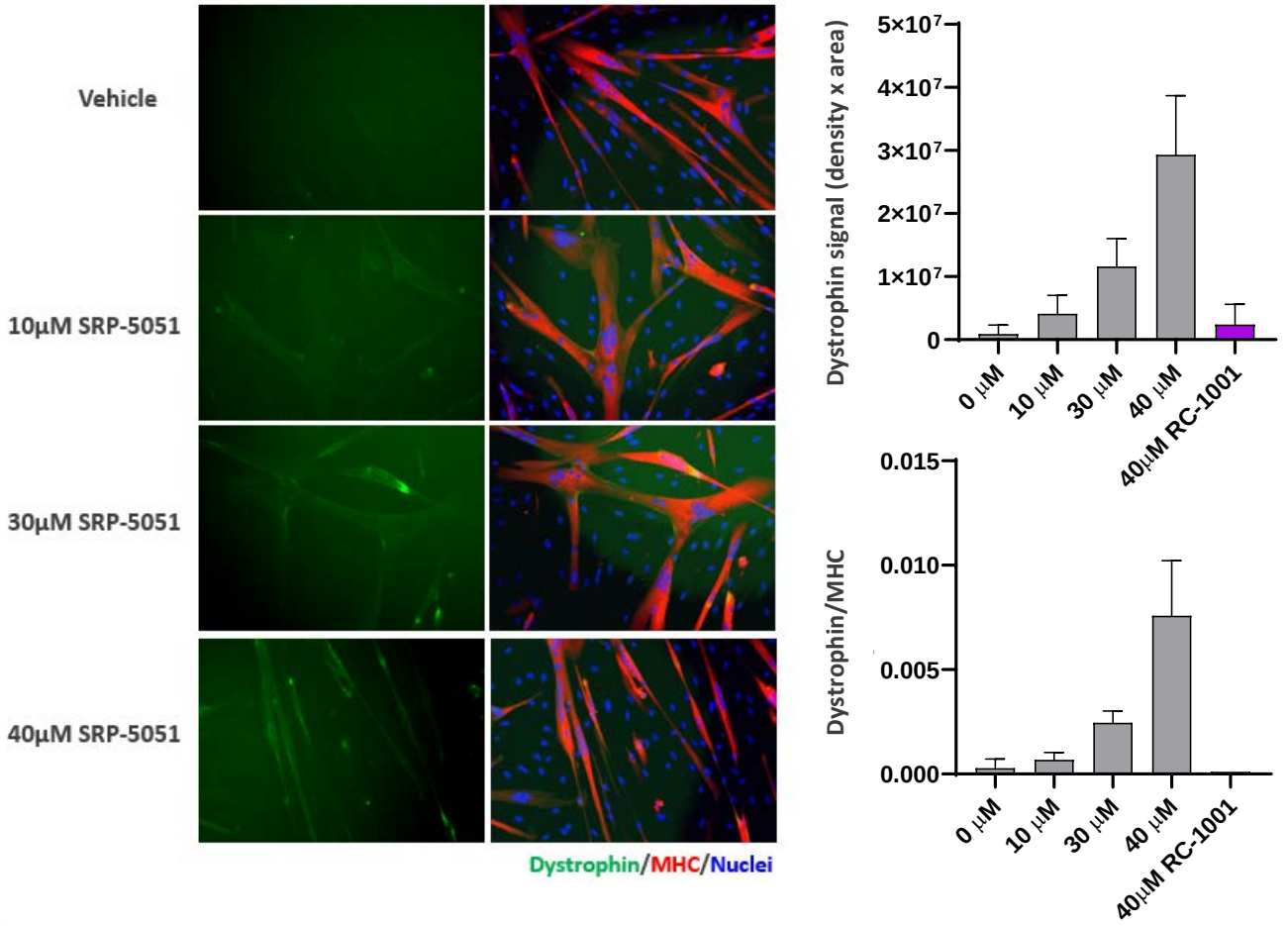
Exon skipping:



Exon skipping EC50 was 5.3 µM ± 1.2 µM in 3 independent experiments

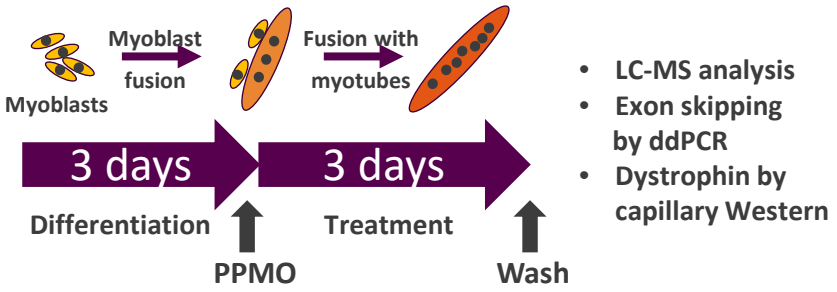
DMD Del52 Cellular Assay: Dystrophin Protein Production With SRP-5051

Dystrophin protein production:

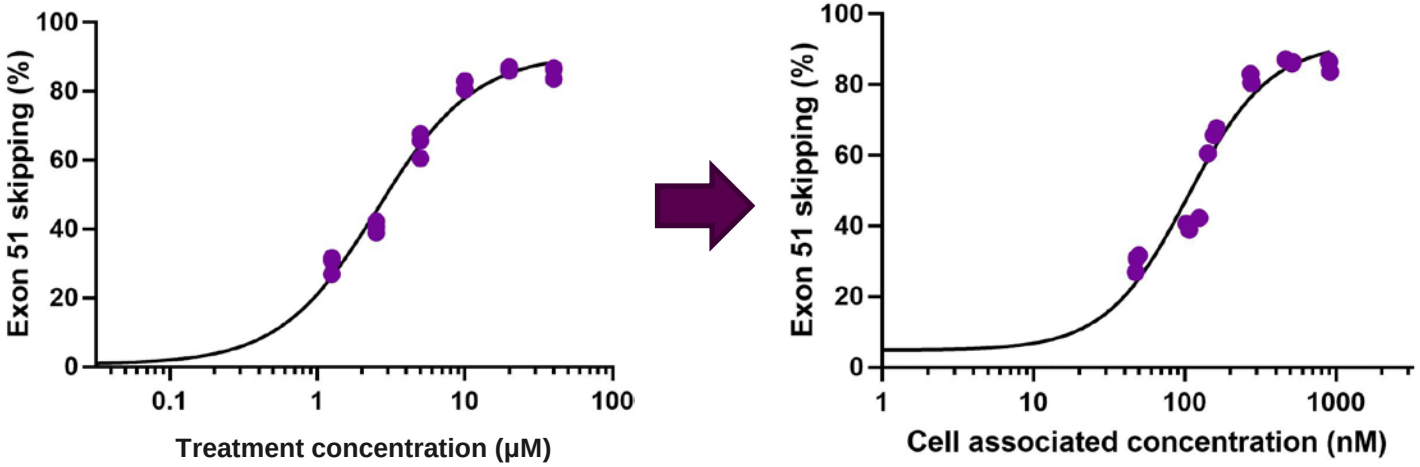


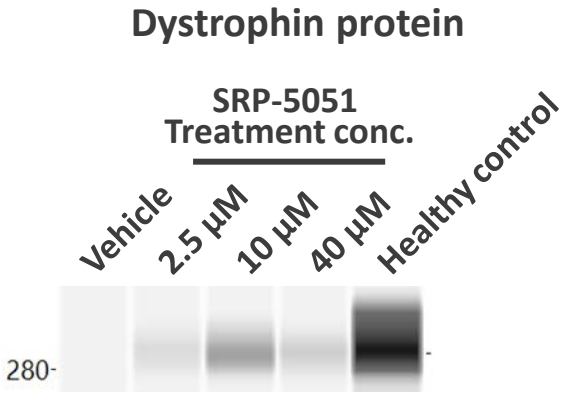
Concentration-dependent dystrophin production was observed at >10 µM

SRP-5051 Cellular Concentration Directly Measured by Liquid Chromatography-Mass Spectrometry



- LC-MS analysis
- Exon skipping by ddPCR
- Dystrophin by capillary Western





Emax (% exon skipping)	EC50 based on dose (µM)	EC50 based on cell conc (µM)	Ratio
85%	3	0.1	30

Cellular concentrations of 100-1000 nM SRP-5051 drive exon skipping and dystrophin protein production in DMD patient-derived myotubes

Conclusions

- DMD patient-derived cellular assays were developed to enable, for the first time, quantitative in vitro measurement of exon skipping and dystrophin protein production mediated by gymnotically delivered PPMOs
- SRP-5051 efficacy, measured by exon skipping and dystrophin protein production, was demonstrated in these patient-derived myotubes
  - Cell-associated concentrations combined with exon skipping and dystrophin production were used to determine the cellular potency of SRP-5051 in the DMD patient myotubes
- These rapid, high-content assays provide a platform approach for elucidating the pharmacology of PPMOs in a disease-relevant model
- These studies further support the clinical investigation of SRP-5051
  - A phase 2 multiple-ascending-dose study is currently ongoing (NCT04004065)

Questions?

Please direct any questions you may have to the Sarepta Medical Information team at medinfo@sarepta.com