FORCE™ PLATFORM DELIVERS EXON SKIPPING PMO, LEADS TO DURABLE INCREASES IN DYSTROPHIN PROTEIN IN *mdx* MICE, AND IS WELL TOLERATED IN NHPs

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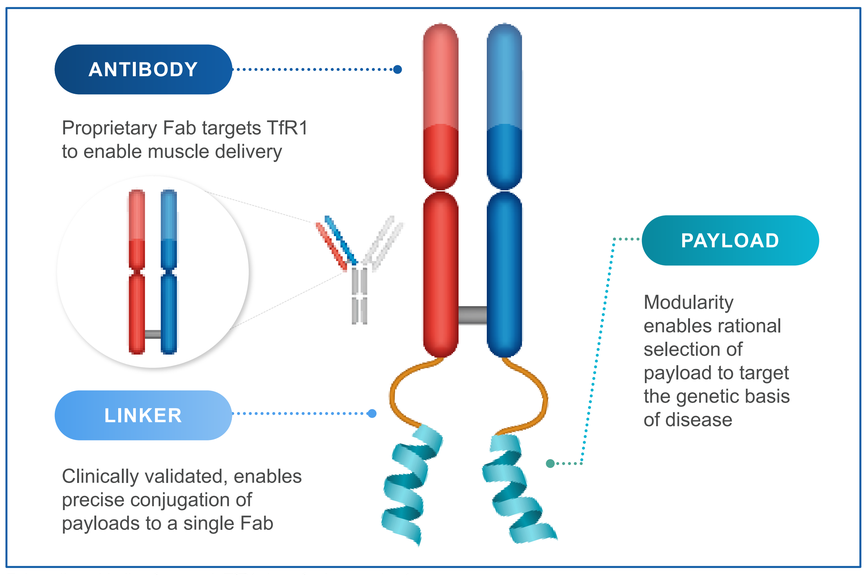
BACKGROUND

- Duchenne muscular dystrophy (DMD) is caused by mutations in the *DMD* gene resulting in the loss of functional dystrophin protein production
- Current therapies for DMD use phosphorodiamidate morpholino oligomers (PMOs) to induce exon skipping in the dystrophin pre-mRNA, enabling the translation of a shortened but functional dystrophin protein
- However, the efficacy of currently approved therapeutic approaches using systemic administration of exon skipping PMO chemistry is limited by poor muscle delivery secondary to inefficient cellular uptake and rapid renal clearance.[1-5] Thus, there is a need for new strategies to efficiently deliver exon-skipping oligonucleotides to muscle in patients with DMD
- The FORCE™ platform was developed to overcome the limitations of oligonucleotide delivery to muscle. It consists of an oligonucleotide payload conjugated to an antigen-binding fragment (Fab) targeting the extracellular region of the human transferrin receptor 1 (hTfR1) which is expressed in muscles.[6-8] This allows for the rational selection of payloads to target the genetic basis of disease
- In this study, we used the FORCE platform to deliver an exon-skipping PMO to muscle in *mdx* mice, an established preclinical model of DMD, wild type cynomolgus monkeys, and myotubes isolated from patients with DMD

Figure 1. Dyne FORCE™ Platform: Modern Oligo Therapeutics for Muscle Diseases

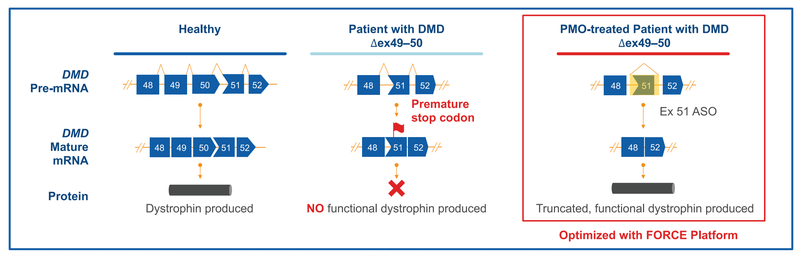


Fab, antigen-binding fragment; TfR1, transferrin receptor 1.

METHODS

- FORCE-M23D is a Fab-conjugated PMO designed to skip exon 23 of the mouse *Dmd* pre-mRNA. *Mdx* mice were administered with a single dose of FORCE-M23D containing the equivalent of 10 or 30 mg/kg PMO. Muscle PMO concentration, exon 23 skipping, and dystrophin protein were measured at multiple timepoints
- To determine translatability of mouse findings to higher species, Dyne's clinical candidate, DYNE-251, a Fab-conjugated PMO designed to skip exon 51, was evaluated for its ability to induce exon skipping in myotubes from patients with DMD amenable to exon 51 skipping and in wild-type non-human primates
- A GLP toxicology study was conducted in non-human primates (NHPs) to assess the safety profile of DYNE-251

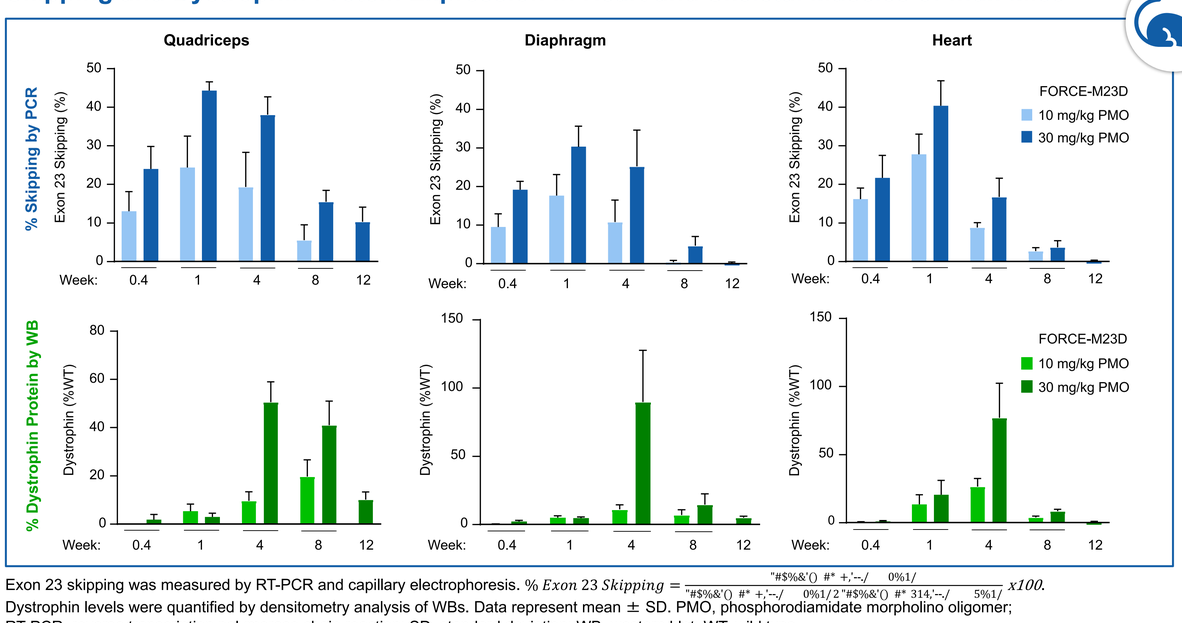
Figure 2. DYNE-251 Targets the Genetic Basis of DMD in Patients Amenable to Exon 51 Skipping



PMO, phosphorodiamidate morpholino oligomers; ASO, antisense oligonucleotide; DMD, Duchenne muscular dystrophy.

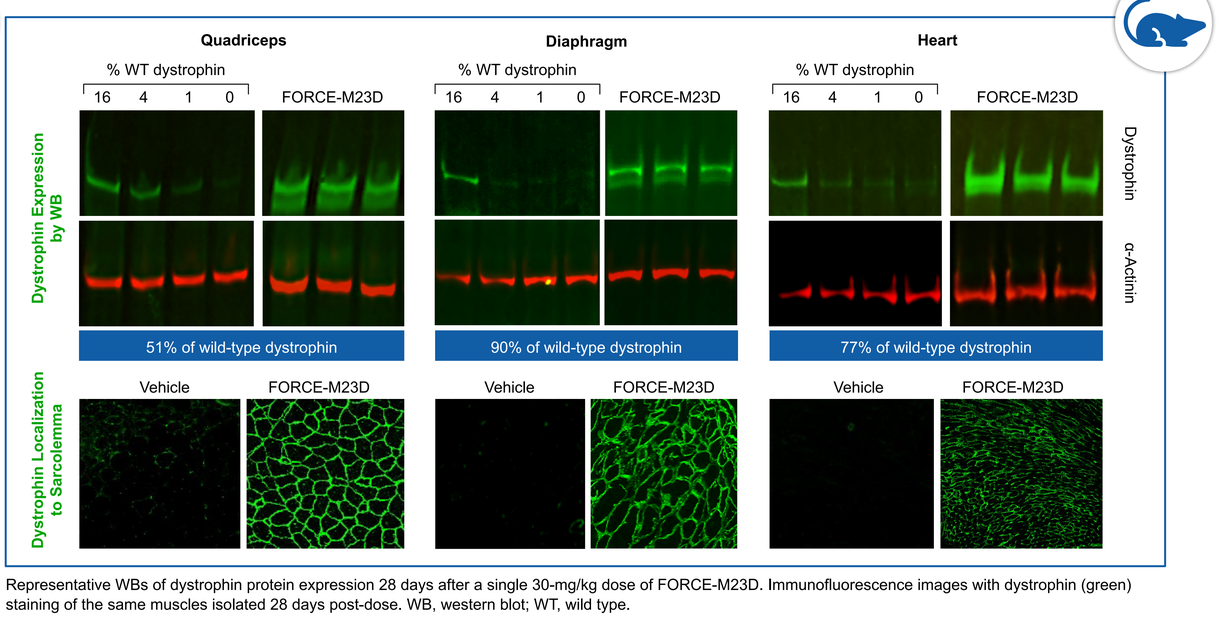
RESULTS

Figure 3: A Single Dose of FORCE-M23D Induces Dose-Dependent *Dmd* Exon 23 Skipping and Dystrophin Protein Expression in Heart and Skeletal Muscles of *mdx* Mice



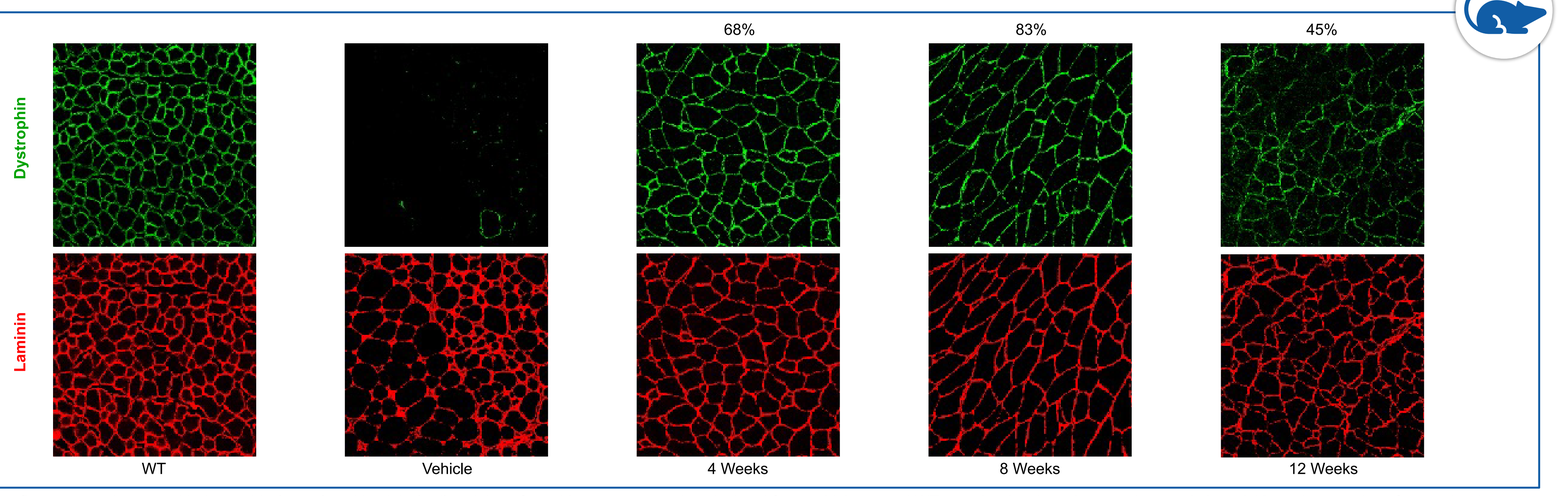
Exon 23 skipping was measured by RT-PCR and capillary electrophoresis. $\%\ Exon\ 23\ Skipping = \frac{\text{"\#\$\%\&'() \#* +,'--./ 0\%1/}}{\text{"\#\$\%\&'() \#* +,'--./ 0\%1/2"\#\$\%\&'() \#* 314,'--./ 5\%1/}}\ x100.$ Dystrophin levels were quantified by densitometry analysis of WBs. Data represent mean ± SD. PMO, phosphorodiamidate morpholino oligomer; RT-PCR, reverse transcription polymerase chain reaction; SD, standard deviation; WB, western blot; WT, wild type.

Figure 4: Single Dose of FORCE-M23D Achieves Durable Dystrophin Expression and Localization to Sarcolemma in Cardiac and Skeletal Muscle of *mdx* Mice



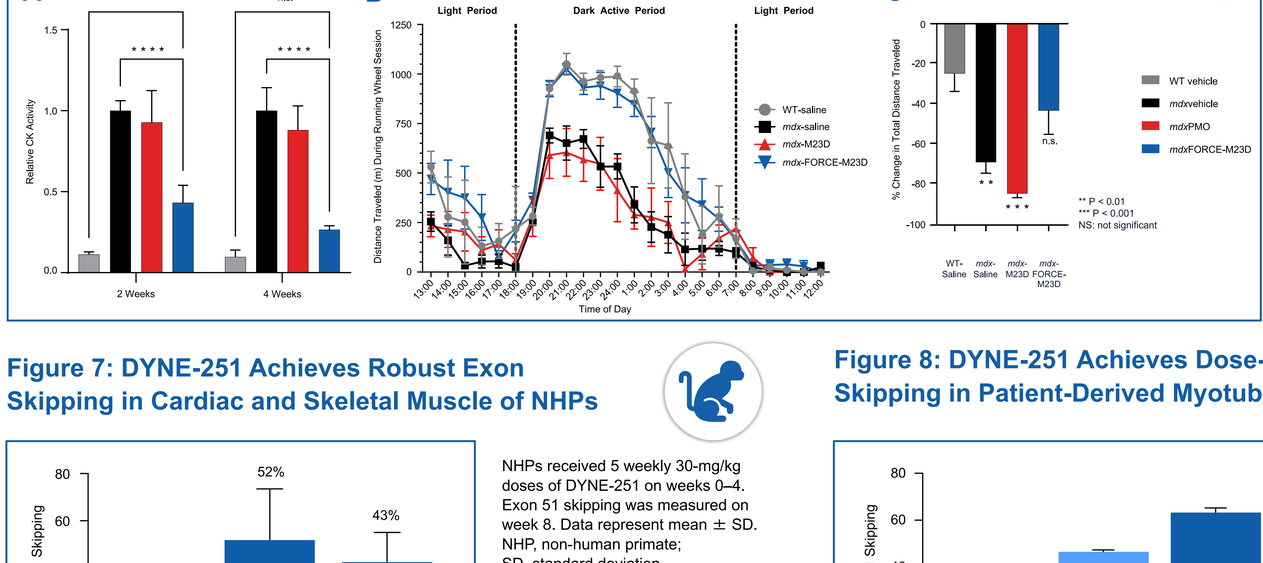
Representative WBs of dystrophin protein expression 28 days after a single 30-mg/kg dose of FORCE-M23D. Immunofluorescence images with dystrophin (green) staining of the same muscles isolated 28 days post-dose. WB, western blot; WT, wild type.

Figure 5: Up to 80% Dystrophin-Positive Fibers in Quadriceps Following Treatment With a Single Dose of FORCE-M23D



Immunofluorescence images with dystrophin (green) and laminin (red) staining of quadriceps cross-sections isolated from vehicle-treated WT or *mdx* mice 4 weeks post-dose or 4, 8, and 12 weeks post-dose from *mdx* mice treated with 30 mg/kg FORCE-M23D. WT, wild type.

Figure 6: Treatment With FORCE-M23D Leads to Improved Functional Outcomes in *mdx* Mice

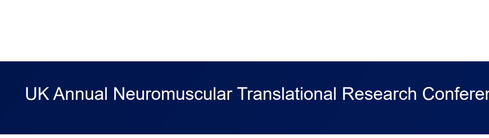


Functional assessments performed following administration of 5-week-old WT or *mdx* mice with vehicle or *mdx* mice injected with 30 mg/kg unconjugated PMO or FORCE-M23D containing the equivalent of 30 mg/kg PMO. (A) Serum CK activity measured 2 or 4 weeks post-dose. (B) Total distance travelled on a running wheel for an uninterrupted 24-hour period 4 weeks post-dose. (C) Percent change in total distance travelled in an open field after hind limb fatigue challenge 2 weeks post-dose. Data are expressed as mean ± SD. $^{*}P \le .05$; $^{**}P \le .01$; $^{***}P \le .001$; $^{****}P \le .0001$. CK, creatine kinase; n.s., not significant; PMO, phosphorodiamidate morpholino oligomer; WT, wild type; SD, standard deviation.

Figure 7: DYNE-251 Achieves Robust Exon Skipping in Cardiac and Skeletal Muscle of NHPs

NHPs received 5 weekly 30-mg/kg doses of DYNE-251 on weeks 0–4. Exon 51 skipping was measured on week 8. Data represent mean ± SD. NHP, non-human primate; SD, standard deviation.

Figure 8: DYNE-251 Achieves Dose-Dependent Exon Skipping in Patient-Derived Myotubes With DMD



Exon 51 skipping was in Myotubes of Patients with DMD after exposure to DYNE-251 for 10 days. Data represent mean ± SD. DMD, Duchenne muscular dystrophy; SD, standard deviation.

DYNE-251 Demonstrated a Favorable Safety Profile in a 5-Week GLP Toxicology Study in NHPs[1]

- No dose limiting toxicity observed after five weekly doses up to a maximally feasible dose
- No changes in cardiac, respiratory, neurologic, or ophthalmic endpoints
- No effect on kidney function
- No effect on liver function
- No effect on coagulation
- NOAEL was identified at the highest dose tested

NHP, non human primate; NOAEL, NOAEL: No-observed-adverse-effect level
1. Based on conclusions of report from third-party CRO

CONCLUSIONS

- In the *mdx* mouse model of DMD, FORCE-M23D achieved: dose-dependent, robust, and durable exon skipping and dystrophin expression as well as long-lasting dystrophin sarcolemma localization and improvement in functional outcomes
- DYNE-251 demonstrated robust exon skipping in NHPs' cardiac and skeletal muscles and in myotubes bearing a DMD mutation amenable to exon 51 skipping.
- The FORCE platform may provide a promising approach to addressing DMD

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DISCLOSURE INFORMATION All authors are employees of Dyne Therapeutics Inc. and may hold Dyne stock and/or stock options.
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