BUILDING A FORCE™ PLATFORM-BASED DMD FRANCHISE FOR THE TREATMENT OF INDIVIDUALS WITH MUTATIONS AMENABLE TO EXON SKIPPING

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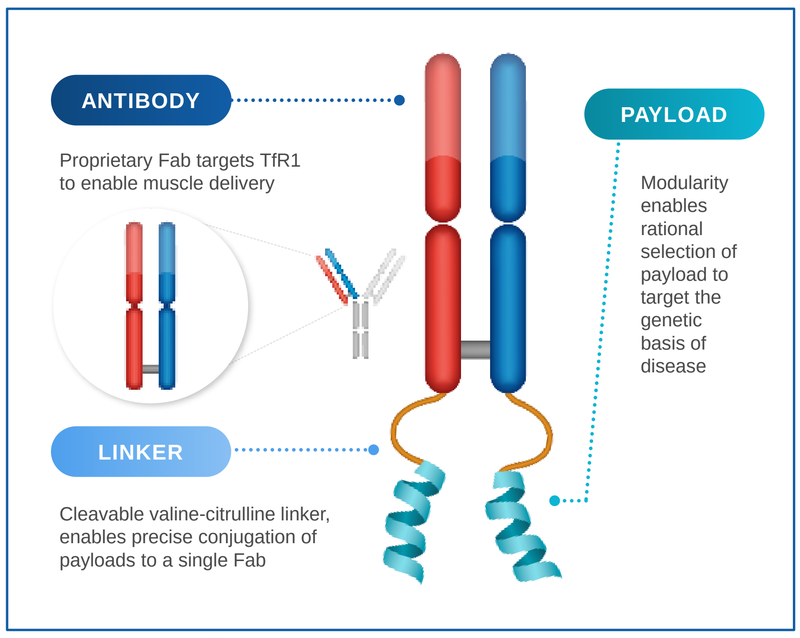
BACKGROUND

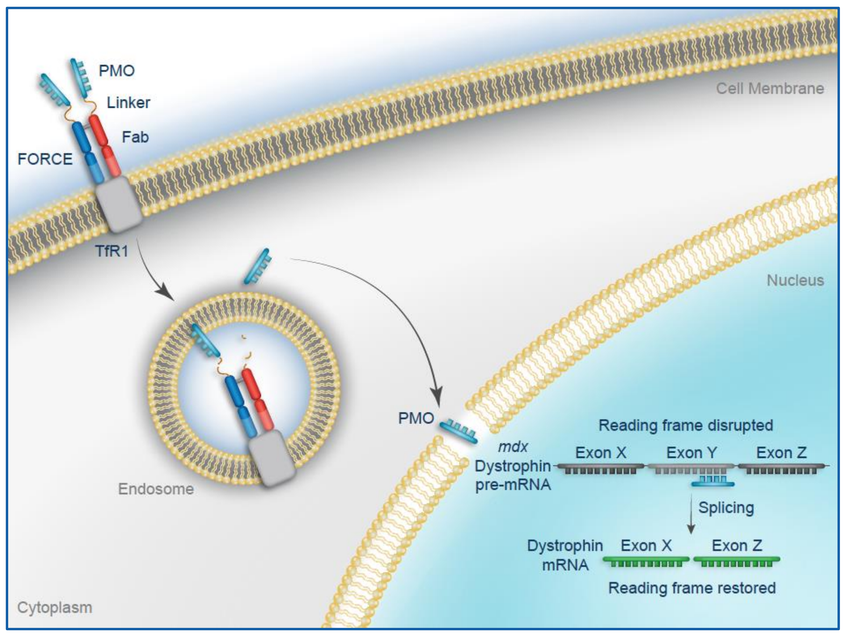
- Duchenne muscular dystrophy (DMD) is caused by mutations in the *DMD* gene resulting in the loss of functional dystrophin protein production
- Current therapies for DMD use phosphorodiamidate morpholino oligomers (PMOs) to induce exon skipping in the dystrophin pre-mRNA, enabling the translation of a shortened but functional dystrophin protein
- However, success of this strategy has been hampered by insufficient distribution to muscle.[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 *mdx* mice, non-human primates (NHPs), and myotubes isolated from a patient with DMD

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



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

Figure 2. PMOs Target the Genetic Basis of DMD in Patients Amenable to Exon Skipping

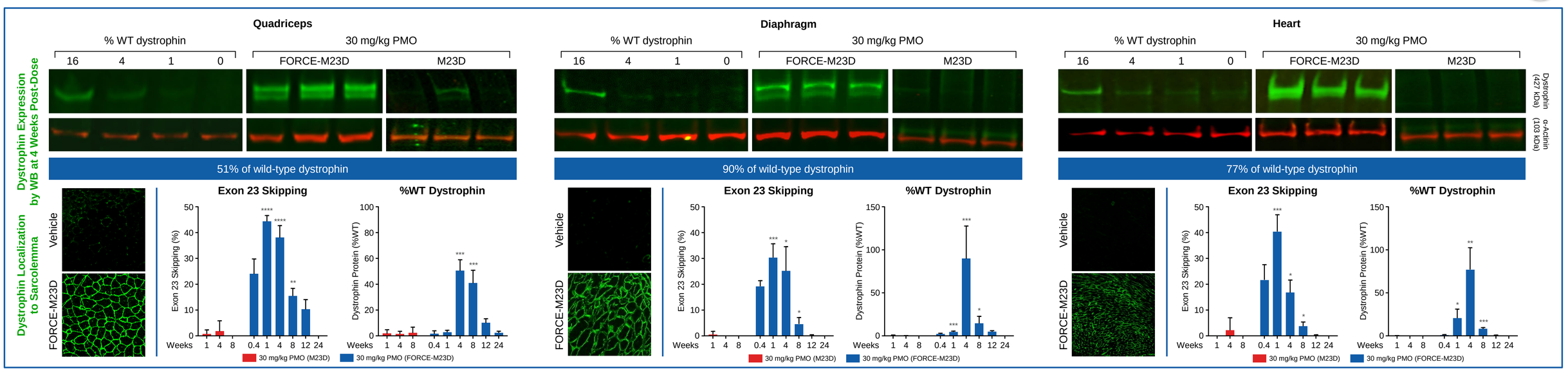


METHODS

- FORCE-M23D is a mouse-specific Fab-PMO conjugate 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 30 mg/kg PMO. Muscle PMO concentration, exon 23 skipping, and dystrophin protein were measured at multiple timepoints
- DYNE-251, a Fab-PMO conjugate 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 NHPs
- A GLP toxicology study was conducted in NHPs to assess the safety profile of DYNE-251
- A novel Fab-PMO conjugate containing an exon 53 skipping PMO was also assessed in myotubes derived from a patient with DMD amenable to exon 53 skipping

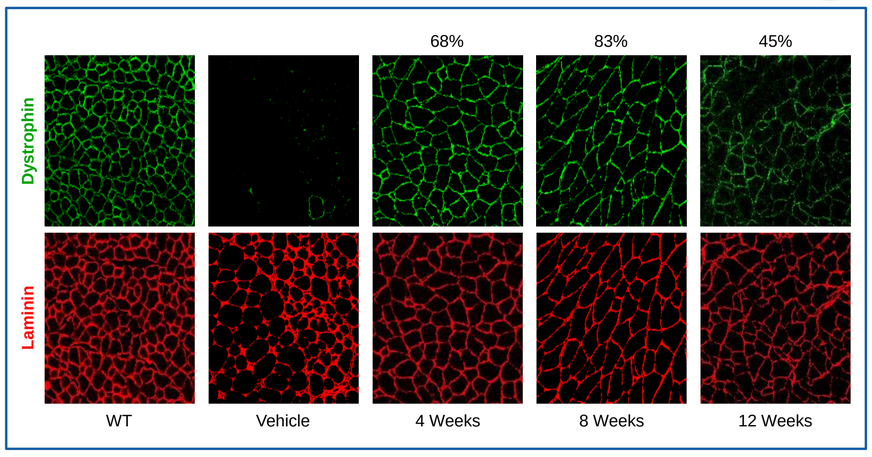
RESULTS

Figure 3: Single Dose of FORCE-M23D, But Not of Unconjugated M23D, Achieves Durable Exon Skipping and Dystrophin Expression in Skeletal and Cardiac Muscle of *mdx* Mice



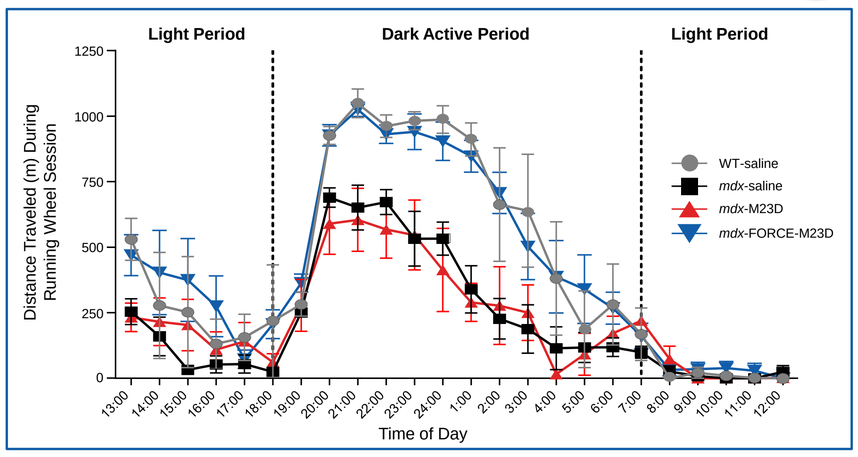
Representative WBs of dystrophin protein expression 28 days after a single 30-mg/kg dose of FORCE-M23D. Immunofluorescence images with dystrophin (green) staining of the same muscles isolated 28 days post-dose. Data are mean ± SD: * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001. SD, standard deviation; WB, western blot; WT, wild type.

Figure 4: Up to 80% Dystrophin-Positive Fibers in Quadriceps Following Treatment With a Single Dose of FORCE-M23D



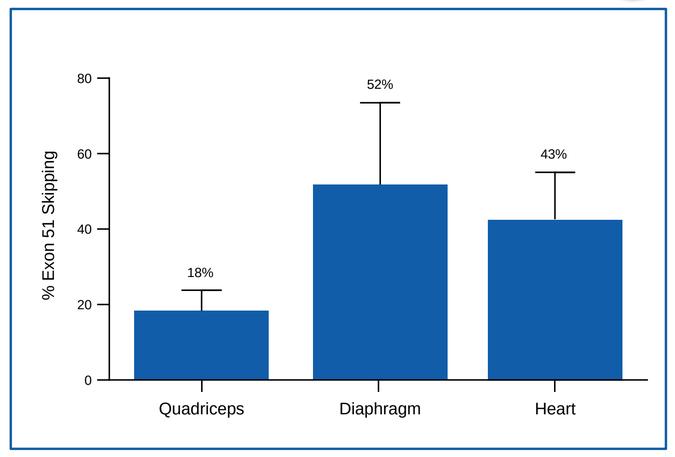
Immunofluorescence images with dystrophin (green) and laminin (red) staining of quadriceps cross-sections isolated from vehicle-treated WT or *mdx* mice 4 weeks post-dose or 4, 8, and 12 weeks post-dose from *mdx* mice treated with 30 mg/kg FORCE-M23D. WT, wild type.

Figure 5: Treatment With FORCE-M23D Leads to Improved Functional Outcomes in *mdx* Mice



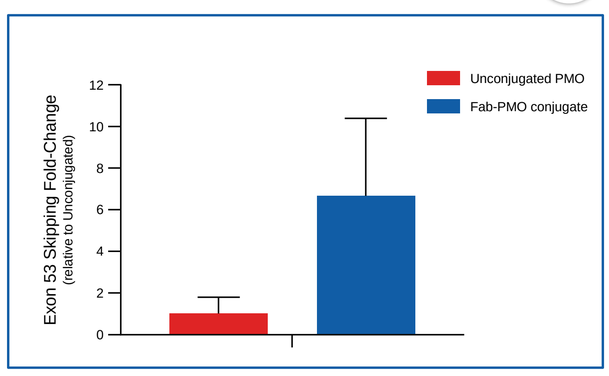
Functional assessments performed following administration of 5-week-old WT or *mdx* mice with vehicle or *mdx* mice injected with 30 mg/kg unconjugated PMO or FORCE-M23D containing the equivalent of 30 mg/kg PMO. Total distance travelled on a running wheel for an uninterrupted 24-hour period 4 weeks post-dose. Overall, data were statistically different in the dark active period between WT-saline and *mdx*-saline (P ≤ .01) and *mdx*-saline and *mdx*-FORCE-M23D (P ≤ .05). Data are mean ± SD.
PMO, phosphorodiamidate morpholino oligomer; WT, wild type; SD, standard deviation.

Figure 6: DYNE-251 Achieves Robust Exon Skipping in Cardiac and Skeletal Muscle of NHPs



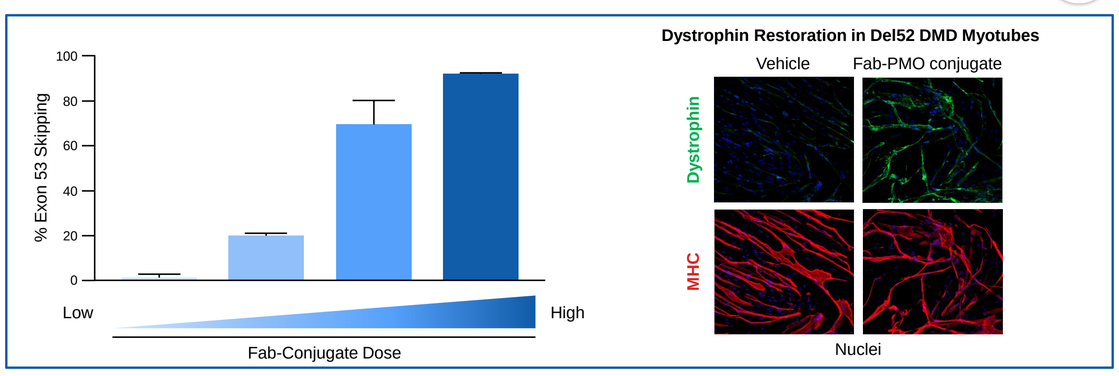
NHPs received 5 weekly 30-mg/kg doses of DYNE-251 on weeks 0–4. Exon 51 skipping was measured on week 8. Data represent mean ± SD. NHP, non-human primate; SD, standard deviation.

Figure 7: Enhanced Efficacy of a Fab-Exon 53 Skipping PMO conjugate in DMD Patient-derived Myotubes



Gymnotic exposure of unconjugated PMO and Fab-PMO conjugate exposure for 10 days. N = 3. Data represent mean ± SD, standard deviation

Figure 8: The Fab-PMO Conjugate Achieves Robust and Dose-Dependent Exon 53 Skipping Leading to Dystrophin Restoration in DMD Patient-derived Myotubes



Gymnotic exposure of Fab-PMO conjugate exposure for 10 days in Del52 myotubes. Immunofluorescence images with dystrophin (green) and myosin heavy chain (MHC; red) staining of Del52 myotubes. N = 3. Data represent mean ± SD, standard deviation

DYNE-251 Demonstrated a Favorable Safety Profile in a 13-Week GLP Toxicology Study in NHPs*

- No dose limiting toxicity observed 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, No-observed-adverse-effect level.
*Based on conclusions of report from third-party CRO

CONCLUSIONS

- In the *mdx* mouse model of DMD, FORCE-M23D induced robust, and durable exon skipping and dystrophin expression as well as long-lasting dystrophin sarcolemma localization and improvement in functional outcomes
- In NHPs, weekly doses of DYNE-251 led to pronounced exon 51 skipping in the heart, diaphragm and quadriceps 8 weeks after the first dose
  - A 13-week GLP toxicology study demonstrated a favorable safety profile
- In addition, a novel *DMD* exon 53 skipping conjugate induced more exon skipping than an unconjugated PMO alone. Moreover, exon 53 skipping observed with the Fab-PMO conjugate was dose responsive and induced restoration of dystrophin protein expression
- Collectively, these data support the use of the FORCE platform for the potential development of novel therapies for individuals living with DMD. DELIVER, a Phase 1/2 clinical study of DYNE-251 in DMD is currently enrolling (NCT05524883).

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DISCLOSURE INFORMATION All authors are employees of Dyne Therapeutics Inc. and may hold Dyne stock and/or stock options. FORCE-M23D and DYNE-251 data were partially presented at the 2022 MDA Clinical and Scientific Conference (Desjardins C.A., et al. Poster 139), at the 2021 Muscle Study Group Annual Meeting (Beskrovnaya O., et al.) and published in Desjardins C et al. *Nucleic Acids Res*. 2022; Aug 10:gkac641. doi: 10.1093/nar/gkac641