FORCE™ Platform Achieves Robust Exon Skipping, Restores Dystrophin at the Sarcolemma and Halts Progression of Fibrosis in the Severe D2-*mdx* Model of DMD

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BACKGROUND

- Therapies for Duchenne muscular dystrophy (DMD) use phosphorodiamidate morpholino oligomers (PMOs) to induce exon skipping in the dystrophin pre-mRNA and enable translation of a shortened, functional dystrophin protein.[1-5] However, PMO efficacy is limited due to insufficient distribution to muscle.
- To overcome the limitations of current PMO-based DMD treatments, we developed the FORCE™ platform, which harnesses the natural expression of transferrin receptor 1 (TfR1) on muscle cells for targeted delivery of oligonucleotides.[6-8]
- In previous work, we demonstrated that FORCE conjugates enhance PMO delivery to muscle, thereby achieving robust dystrophin expression and functional benefit in the BL10-*mdx* mouse model of DMD.[9]
- In this study, we used D2-*mdx* mice to determine the therapeutic potential of the FORCE platform in muscle already damaged by fibrotic degeneration.

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

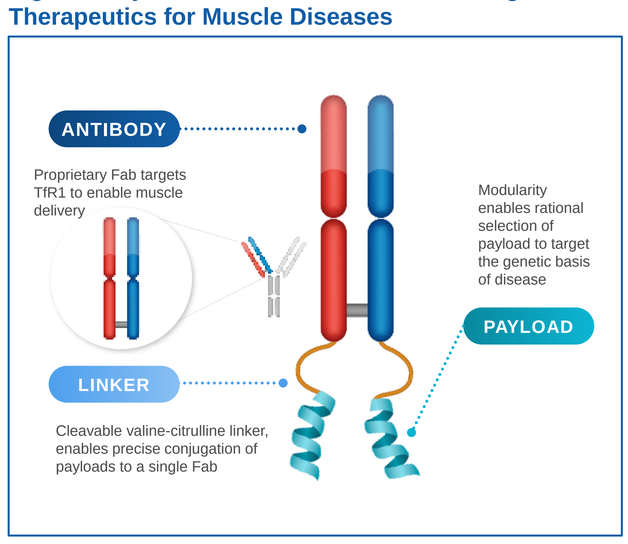
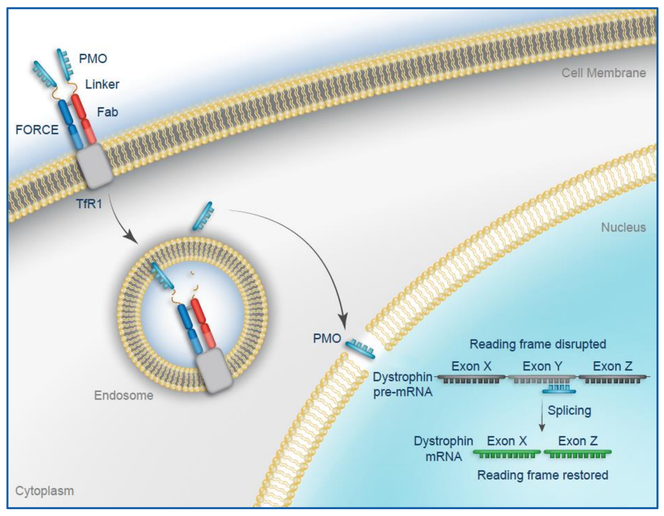


Figure 2. FORCE-Conjugated PMOs Target the Genetic Basis of DMD

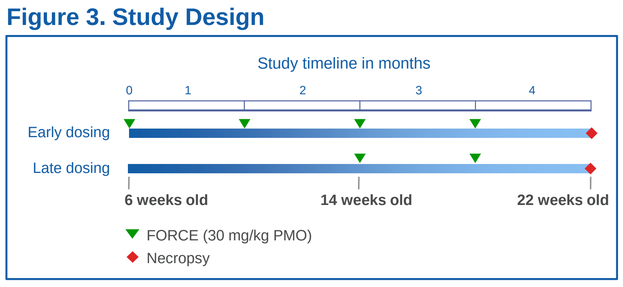


Figures adapted from Desjardins CA, Yao M, Hall J, et al. Enhanced exon skipping and prolonged dystrophin restoration achieved by TfR1-targeted delivery of antisense oligonucleotide using FORCE conjugation in mdx mice. *Nucleic Acids Res*. 2022;50(20):11401-11414. Oxford University Press.
Fab, antigen-binding fragment; PMO, phosphorodiamidate morpholino oligomer; TfR1, transferrin receptor 1.

METHODS

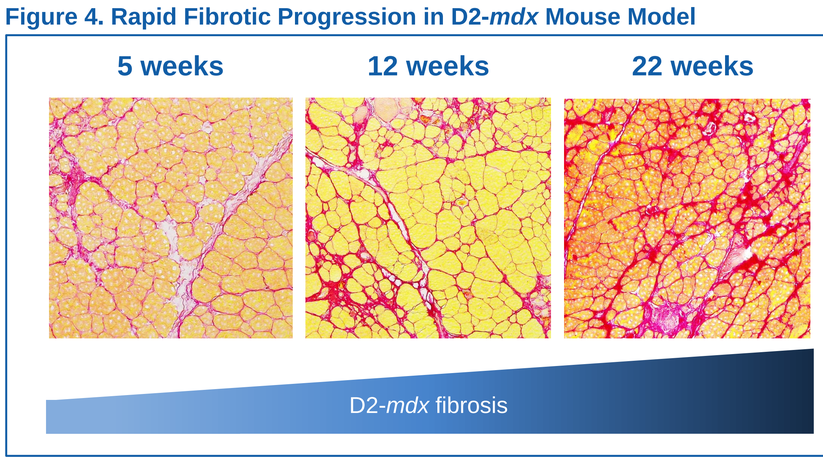
- FORCE-M23D is a mouse-specific FORCE conjugate engineered to deliver the M23D PMO to muscle via TfR1-mediated uptake.
- D2-*mdx* mice were administered FORCE-M23D containing the equivalent of 30 mg/kg PMO or vehicle once monthly. First dose was administered at 6 weeks (early dosing) or 14 weeks (late dosing) of age.
  - D2-*mdx* mice are a severe model of DMD characterized by high levels of fibrosis.
- Muscle histopathology in quadriceps and dystrophin restoration at sarcolemma in quadriceps were evaluated.

Figure 3. Study Design



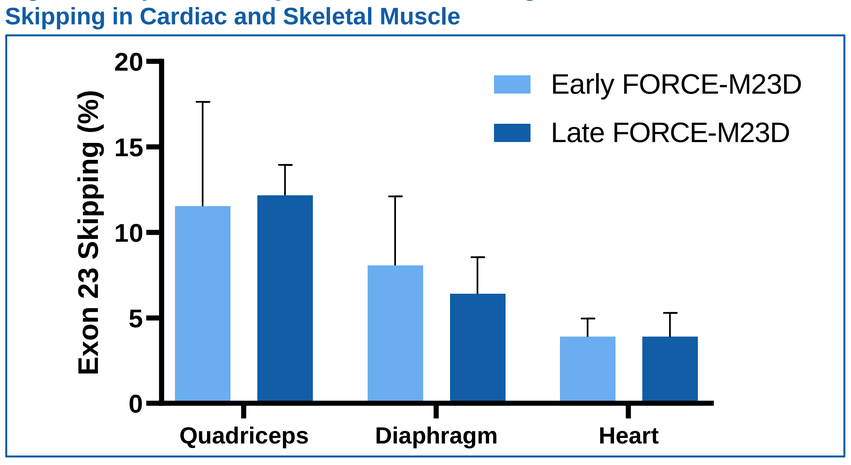
RESULTS

Figure 4. Rapid Fibrotic Progression in D2-*mdx* Mouse Model



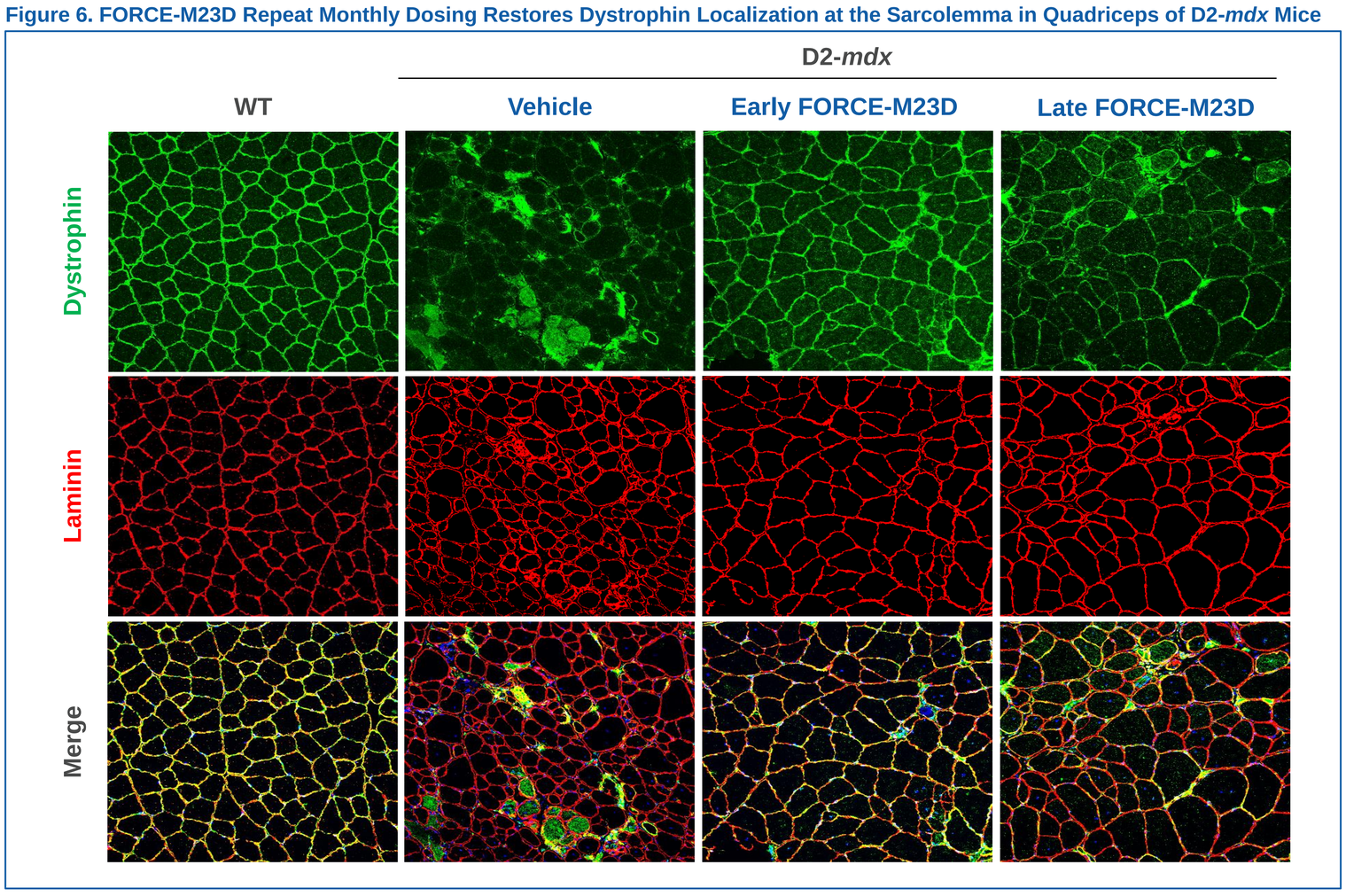
Picrosirius red stain; red staining fibrosis. Cropped images of quadricep sections taken from Zeiss Axioscan Slide Scanner using a 0.8 10x objective.

Figure 5. Repeat Monthly FORCE-M23D Dosing Induces Robust Exon-Skipping in Cardiac and Skeletal Muscle



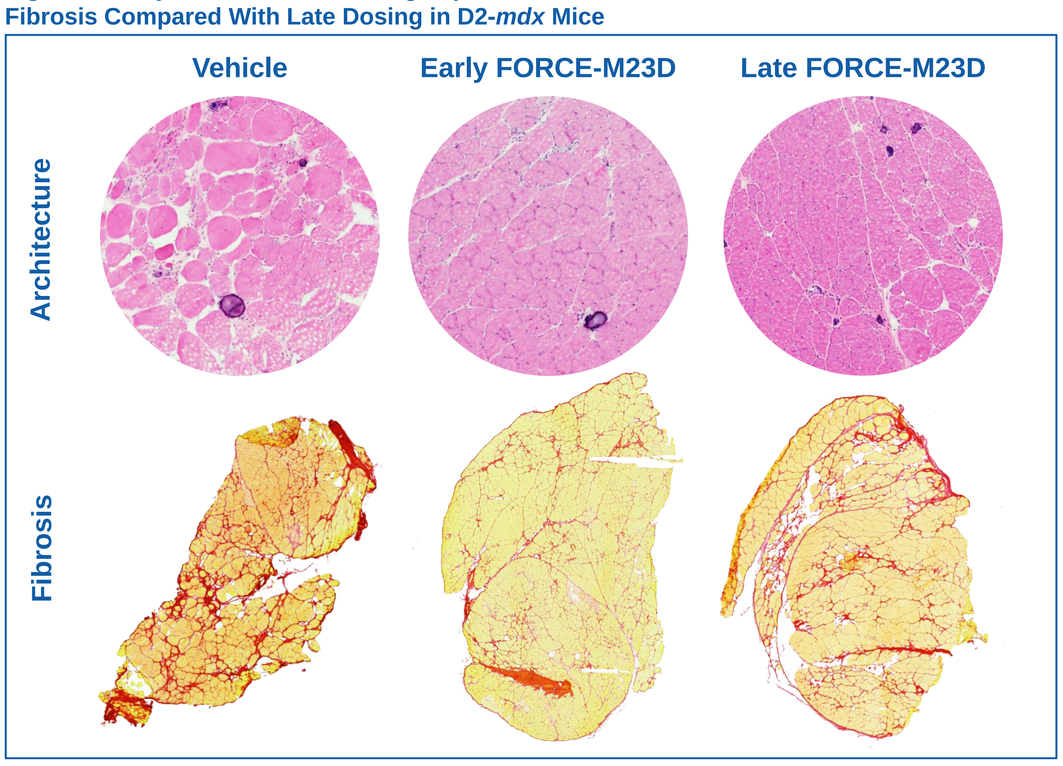
Data are mean ±SD, N = 5.

Figure 6. FORCE-M23D Repeat Monthly Dosing Restores Dystrophin Localization at the Sarcolemma in Quadriceps of D2-*mdx* Mice



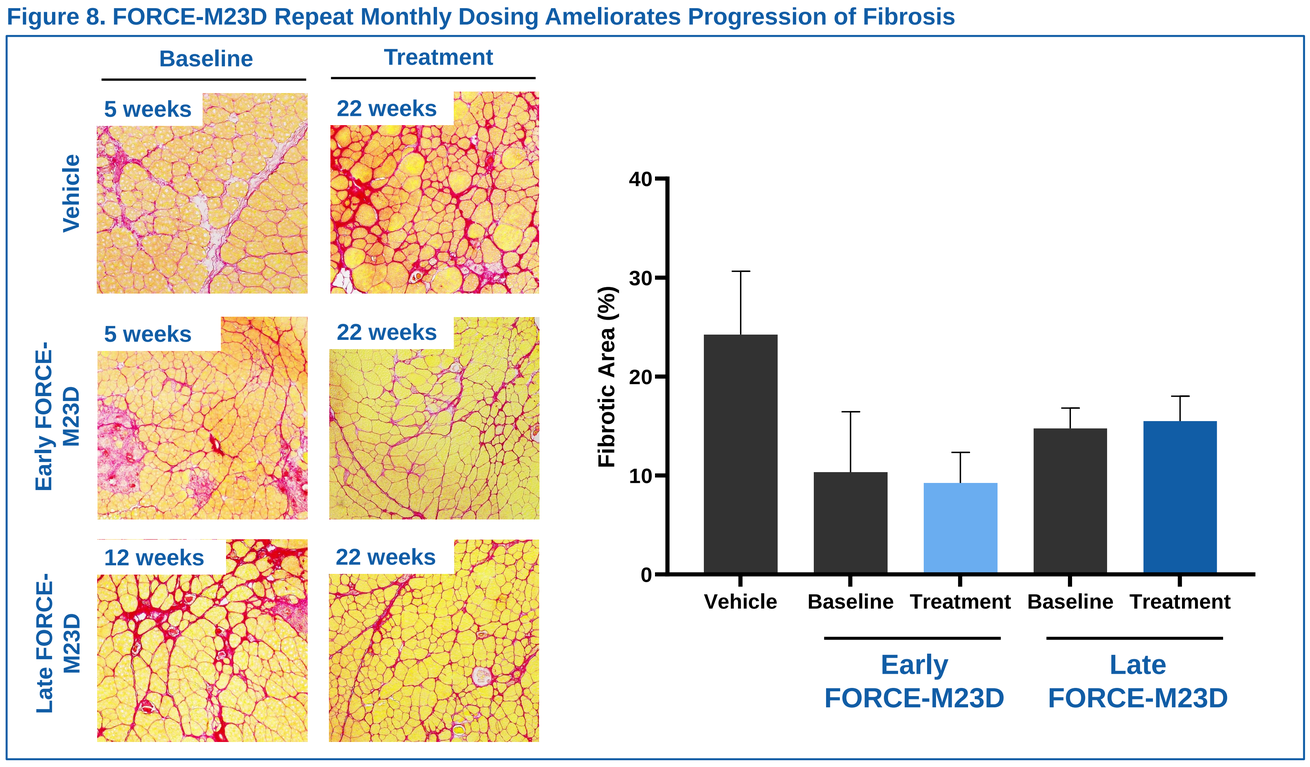
Immunofluorescence images with dystrophin (green) and laminin (red) staining of quadricep cross sections isolated from WT or D2-*mdx* mice at 4 months post treatment with vehicle, 30 mg/kg FORCE-M23D early, or 30 mg/kg FORCE-M23D late. Images were captured on Zeiss LSM800; 0.8 20x objective. WT, wild type.

Figure 7. Early FORCE-M23D Dosing Improves Muscle Architecture and Lowers Level of Fibrosis Compared With Late Dosing in D2-*mdx* Mice



Top: Hematoxylin and eosin stain of quadricep muscle at 10x magnification. Bottom: Picrosirius red stain of quadricep muscle. Picrosirius images were acquired on Zeiss Axioscan Slide Scanner Platform using a 0.8 20x objective; complete quadricep section provided; red staining fibrosis.

Figure 8. FORCE-M23D Repeat Monthly Dosing Ameliorates Progression of Fibrosis



Picrosirius red stain of quadricep muscle. Cropped images taken from Zeiss Axioscan Slide Scanner using a 0.8 10x objective; red staining fibrosis. Data are mean ±SD, N = 5.

CONCLUSIONS

- Repeat monthly dosing with FORCE-M23D achieved robust dystrophin restoration to the sarcolemma of skeletal muscles and improved muscle morphology of D2-mdx mice.
- Importantly, starting FORCE-M23D treatment at 6 weeks of age led to lower deposition of fibrotic tissue compared with initiation at 14 weeks of age, indicating that earlier treatment with FORCE conjugates may lead to greater benefit.
- These data will inform interpretation of the response of a heterogenous DMD human population to DYNE-251, an exon 51–skipping FORCE conjugate currently being investigated in the Phase 1/2 DELIVER trial of patients with DMD with mutations amenable to exon 51 skipping (NCT05524883).

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ACKNOWLEDGEMENTS: Medical writing and editorial support were provided by Symbiotix, LLC, and funded by Dyne Therapeutics, Inc. **DISCLOSURE INFORMATION:** All authors are employees of Dyne Therapeutics, Inc. and may hold Dyne stock and/or stock options.