FORCE™ Platform for Targeted Delivery of Oligonucleotide Therapeutics in Muscle Diseases

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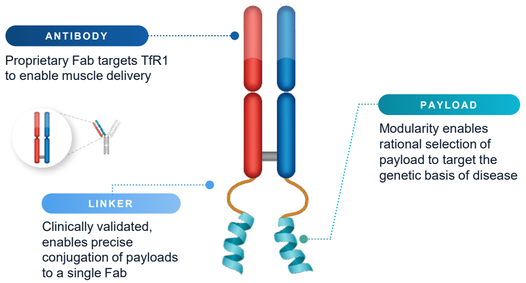
BACKGROUND

- Lack of targeted delivery of oligonucleotide therapeutics to muscle tissues remains a challenge in the treatment of muscle diseases. The FORCE™ platform, designed to overcome these limitations, consists of an oligonucleotide payload conjugated to an antigen-binding fragment (Fab) targeting the extracellular region of the human transferrin receptor (hTfR)1 which is expressed in muscles[1-3]. We used the FORCE™ platform to deliver therapeutic oligonucleotides to muscle in preclinical models of Duchenne muscular dystrophy (DMD) and myotonic dystrophy type 1 (DM1)

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

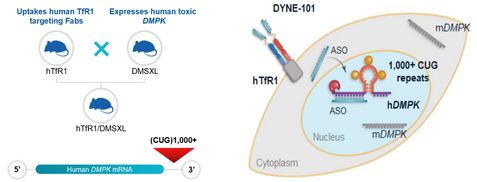


- DMD, caused by mutations in the *DMD* gene, results in the loss of functional dystrophin protein production. Success of therapies using phosphorodiamidate morpholino oligomer (PMO) has been hampered by insufficient distribution of PMO to muscle[4-8]
- FORCE-M23D, a mouse-specific Fab-PMO conjugate designed to skip exon 23, was evaluated in *mdx* mice, a model of DMD caused by a nonsense mutation in exon 23. Additionally, Dyne's clinical candidate, DYNE-251, a Fab-conjugated PMO designed to skip exon 51, was evaluated in non-human primates (NHPs) and DMD patient myotubes
- DM1 is caused by abnormal expansion of a region of the dystrophia myotonica protein kinase (*DMPK*) gene[9]. There are no approved therapies for DM1[10]
- DYNE-101 is a Fab-conjugated antisense oligonucleotide designed to target toxic human nuclear *DMPK* for RNAseH-mediated degradation in DM1

METHODS

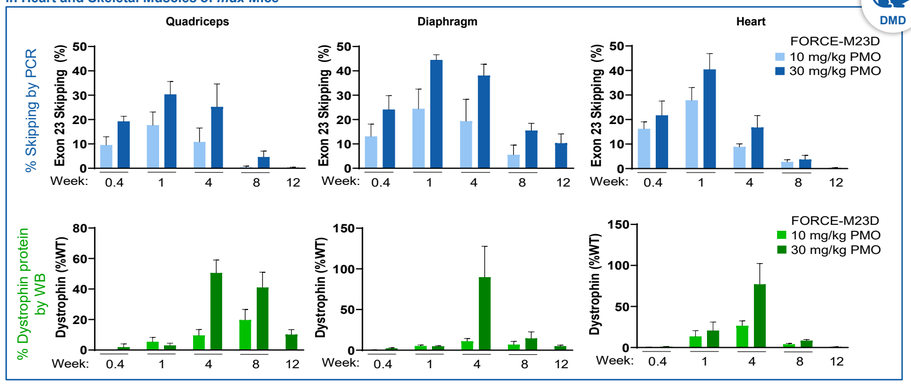
- To analyze exon skipping and dystrophin restoration, 5-week-old male *mdx* mice were injected via tail vein with a single IV dose of vehicle or FORCE-M23D containing the equivalent of 10 or 30 mg/kg PMO. DYNE-251 was evaluated for its ability to induce exon skipping in NHPs and myotubes isolated from patients with DMD
- DYNE-101 was assessed for its ability to knock down toxic human *DMPK* RNA in hTfR1/DMSXL mice, a novel model expressing human TfR1 (hTfR1) and a human *DMPK* with > 1000 CTG repeats (DMSXL). hTfR1/DMSXL mice were administered 10 mg/kg DYNE-101 or vehicle via tail injections on days 0 and 7. *DMPK* RNA, foci, and splicing were assessed on day 28

Figure 2. hTfR1/DMSXL Mice are an Innovative Model to Evaluate PD of DYNE-101 by Measuring Toxic Human Nuclear *DMPK* KD



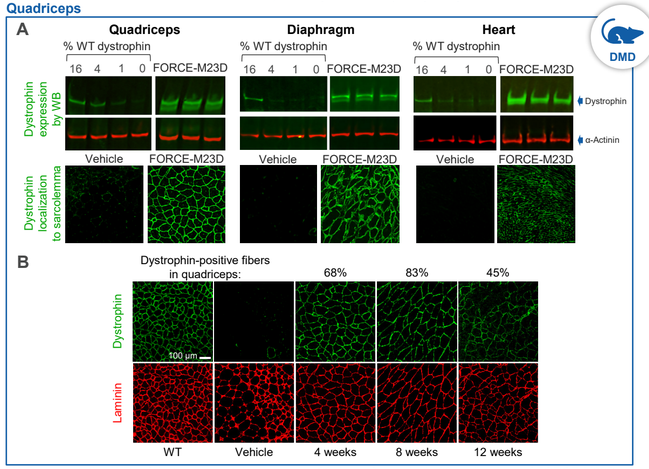
RESULTS

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



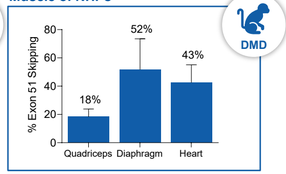
Exon 23 skipping was measured by RT-PCR and capillary electrophoresis. $\%\ Exon\ 23\ Skipping = \frac{Molarity\ of\ Skipped\ Band}{Molarity\ of\ Skipped\ Band + Molarity\ of\ Unskipped\ band} * 100$. Dystrophin levels were quantified by densitometry analysis of western blots. Data represent mean ± SD. RT-PCR, reverse transcription polymerase chain reaction; WT, wild type

Figure 4. A Single Dose of FORCE-M23D Achieves Robust and Durable Dystrophin Expression in Cardiac and Skeletal Muscle of *mdx* Mice, With up to 80% Dystrophin-positive Fibers in the Quadriceps



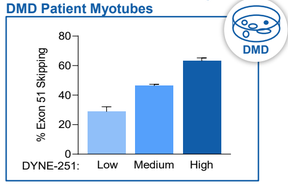
(A) Representative WBs of dystrophin protein expression 28 days after a single 30-mg/kg dose of FORCE-M23D. Immunofluorescence images with dystrophin (green) staining of the same muscles isolated 28 days post-dose. (B) Immunofluorescence images with dystrophin (green) and laminin (red) staining of quadriceps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

Figure 5. 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.

Figure 6. DYNE-251 Achieves Dose-Dependent Exon Skipping in DMD Patient Myotubes

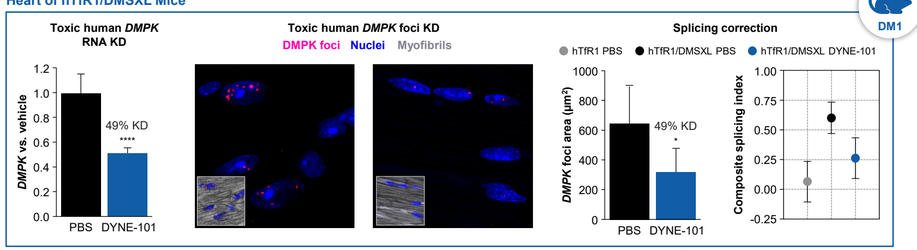


Exon 51 skipping was measured in DMD patient myotubes after exposure to DYNE-251 for 10 days. Data represent mean ± SD.

DYNE-251 TOLERABILITY

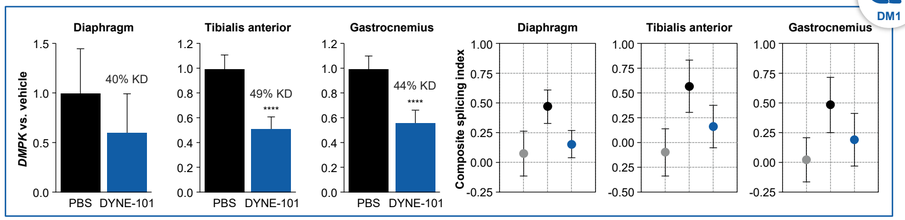
- DYNE-251 showed a favorable safety profile in a 5-week GLP toxicology study in NHPs

Figure 7. DYNE-101 Delivers Sustained Toxic Human *DMPK* RNA KD and Foci Reduction, Leading to Splicing Correction in the Heart of hTfR1/DMSXL Mice



DMPK KD measured by qRT-PCR, representative images from *in situ* hybridization chain reaction in heart tissues with quantification on FIJI software, composite splicing index of *Ldb3* exon (E)11, *Mbnl2* exon E6, and *Nfix* E7 mis-splicing measured by qRT-PCR. Data are mean ± SD; n = 7; *P < .05; ****P < .0001, by *t*-test. *DMPK*, dystrophia myotonica protein kinase; hTfR1, human transferrin receptor 1; KD, knockdown; PBS, phosphate-buffered saline; qRT-PCR, quantitative reverse transcription polymerase chain reaction; SD, standard deviation

Figure 8. DYNE-101 Delivers Sustained Toxic Human *DMPK* RNA KD, Leading to Splicing Correction in Skeletal Muscles of hTfR1/DMSXL Homozygous Mice



DMPK KD measured by qRT-PCR; composite splicing index[5] of *Bin1* E11, *Insr* E11, *Ldb3* E11, *Mbnl2* E5, *Mbnl2* E6, *Nfix* E7, and *Ttn* E313 mis-splicing measured by qRT-PCR. Data are mean ± SD; n = 4-7; P < .0001, by *t*-test. *DMPK*, dystrophia myotonica protein kinase; hTfR1, human transferrin receptor 1; KD, knockdown; PBS, phosphate-buffered saline; qRT-PCR, quantitative reverse transcription polymerase chain reaction; SD, standard deviation

DYNE-101 TOLERABILITY

- DYNE-101 was well tolerated in a 13-week GLP toxicology study in NHPs

CONCLUSIONS

- In the *mdx* mouse model of DMD, FORCE-M23D achieved: dose-dependent, robust, and durable exon skipping and dystrophin expression; long-lasting dystrophin sarcolemma localization.
- DYNE-251 demonstrated robust exon skipping in NHP cardiac and skeletal muscles
- The reported data also demonstrate that DYNE-101: reduces toxic human nuclear *DMPK* RNA and foci and corrects splicing defects in the hTfR1/DMSXL model of DM1
- The FORCE platform has the potential to provide an effective, re-dosable, and titratable treatment for patients with muscle diseases, including DM1 and DMD

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DISCLOSURES

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

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