# FORCE™ PLATFORM FOR TARGETED DELIVERY OF OLIGONUCLEOTIDE THERAPEUTICS IN MUSCLE DISEASES

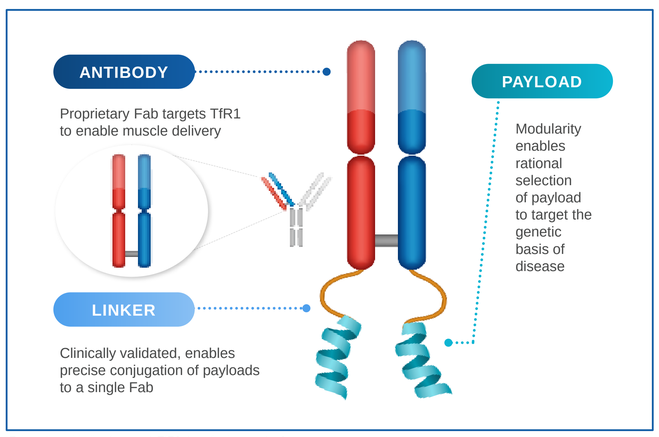
Stefano Zanotti, Cody Desjardins, Tyler Picariello, Sean Spring, Qifeng Qiu, Timothy Weeden, Marc Roy, John Davis, Scott Hilderbrand, John Najim, Oxana Beskrovnaya

Dyne Therapeutics Inc., Waltham, MA, USA

## BACKGROUND

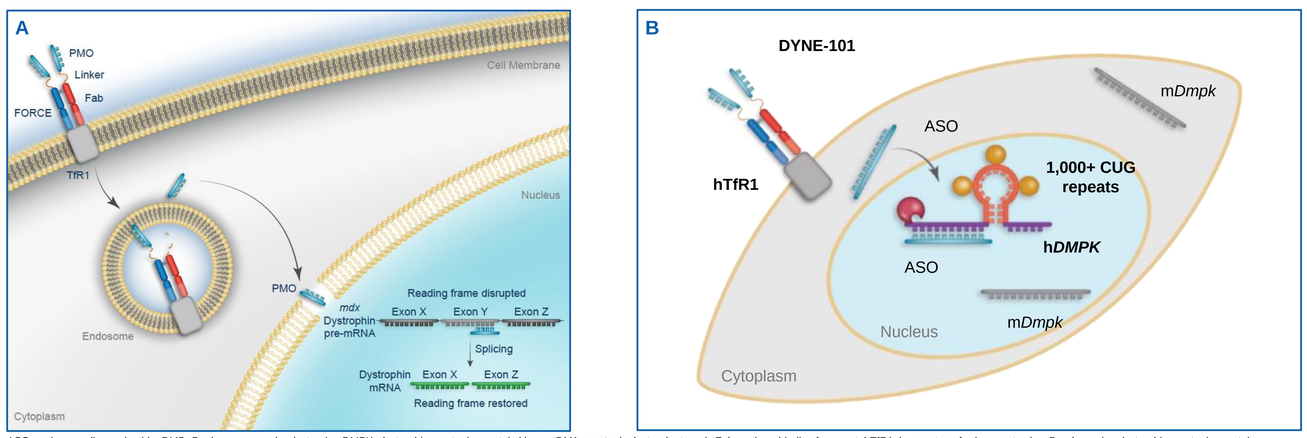
- Lack of targeted delivery of oligonucleotide therapeutics to muscle tissues remains a challenge in the treatment of muscle diseases. The FORCE™ platform, designed to overcome these limitations, consists of an oligonucleotide payload conjugated to an antigen-binding fragment (Fab) targeting the extracellular region of the human transferrin receptor 1 (hTfR1) that 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)
- 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)
- 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 nuclear *DMPK* for RNAseH-mediated degradation in DM1

### 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 (A). DYNE-101 is a Fully Human Fab-ASO Conjugate Designed to Target *DMPK* RNA in DM1 (B)



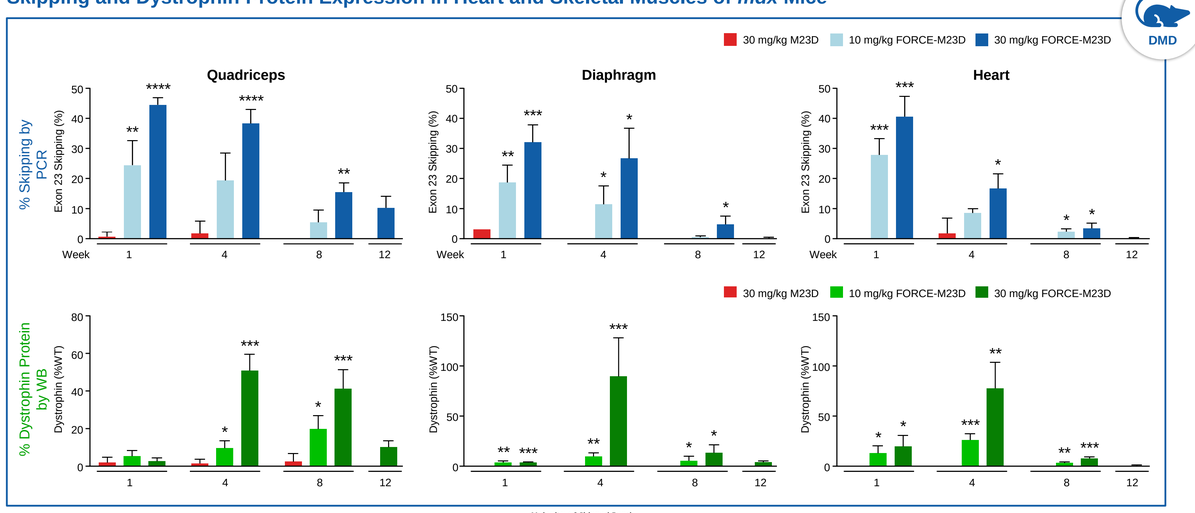

ASO, antisense oligonucleotide; DMD, Duchenne muscular dystrophy; DMPK, dystrophia myotonica protein kinase; DM1, myotonic dystrophy type 1; Fab, antigen-binding fragment; hTfR1, human transferrin receptor 1; mDmpk, murine dystrophia myotonica protein kinase; muscular PMO, phosphorodiamidate morpholino oligomer; TfR1, transferrin receptor 1.

## METHODS

- *Mdx* mice were administered with a single dose of FORCE-M23D containing the equivalent of 30 mg/kg PMO. Exon 23 skipping and dystrophin protein were measured at multiple timepoints. A GLP toxicology study for DYNE-251 was conducted in NHPs
- DYNE-101 was assessed for its ability to knock down toxic human *DMPK* RNA in hTfR1/DMSXL mice, a novel model expressing hTfR1 and a human *DMPK* with > 1,000 CTG repeats (DMSXL). *DMPK* RNA, foci, and splicing were assessed in hTfR1/DMSXL homozygous mice treated on day 0 and day 7 with 10 mg/kg DYNE-101 or with phosphate buffered saline (PBS) and analyzed on day 28. *DMPK* RNA was assessed in hTfR1/DMSXL hemizygous mice treated on month 0 (single dose [SD]) or treated on months 0, 1, 2, and 3 (repeat dose [RD]) with 5 mg/kg DYNE-101 or with vehicle and analyzed on month 1 (SD) or month 4 (RD). *DMPK* RNA was assessed in male cynomolgus monkeys treated on months 0 and 1 (RD) with 10 mg/kg DYNE-101 or with PBS and analyzed on month 2. A GLP toxicology study for DYNE-101 was conducted in NHPs

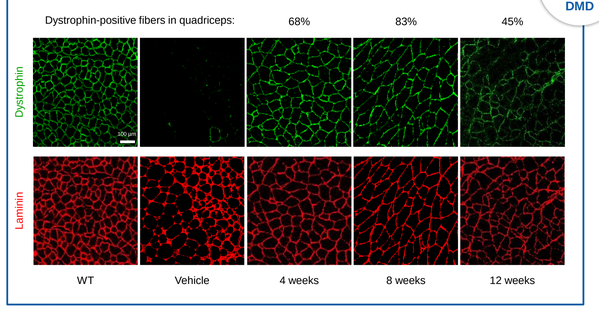
## RESULTS

### Figure 3. A Single Dose of FORCE-M23D, But Not of Unconjugated M23D, Induces Dose-Dependent *DMD* Exon 23 Skipping and Dystrophin Protein Expression in Heart and Skeletal Muscles of *mdx* Mice



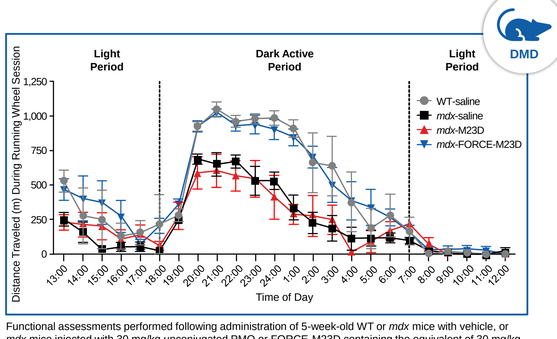

Exon 23 skipping was measured by RT-PCR and capillary electrophoresis. % $Exon\ 23\ Skipping = \frac{Molarity\ of\ Skipped\ Band}{Molarity\ of\ Skipped\ Band + Molarity\ of\ Unskipped\ band} * 100$. Dystrophin levels were quantified by densitometry analysis of Western Blots. Data represent mean ± SD. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001. DMD, Duchenne muscular dystrophy; PCR, polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction; SD, standard deviation; WB, Western Blot; WT, wild type.

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



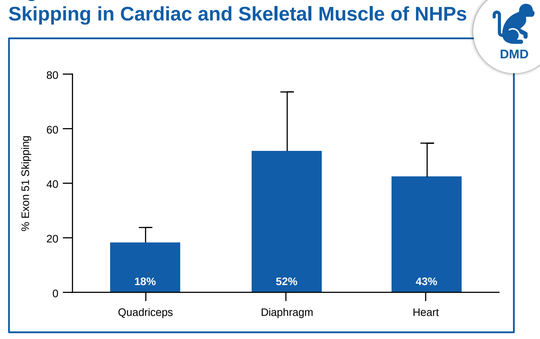

Immunofluorescence images with dystrophin (green) and laminin (red) staining of quadriceps cross-sections isolated from vehicle-treated WT or *mdx* mice 4 weeks post-dose or 4, 8, and 12 weeks post-dose from *mdx* mice treated with 30 mg/kg FORCE-M23D. WT, wild type.

### 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 ($P \leq .01$) and *mdx*-saline and *mdx*-FORCE-M23D ($P \leq .05$). Data are expressed as 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



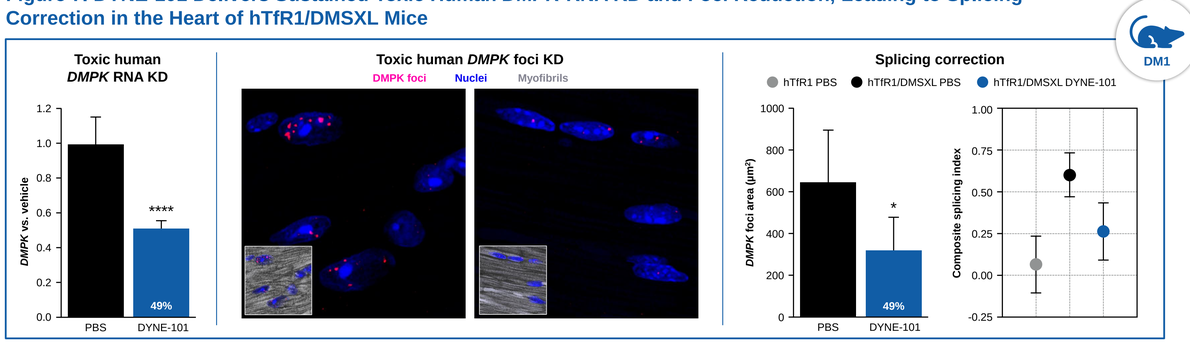

NHPs received 5 weekly 30 mg/kg doses of DYNE-251 on weeks 0–4. Exon 51 skipping was measured on week 8. Data represent mean ± SD. NHP, non human primate; SD, standard deviation.

## DYNE-251 TOLERABILITY & SAFETY

- 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

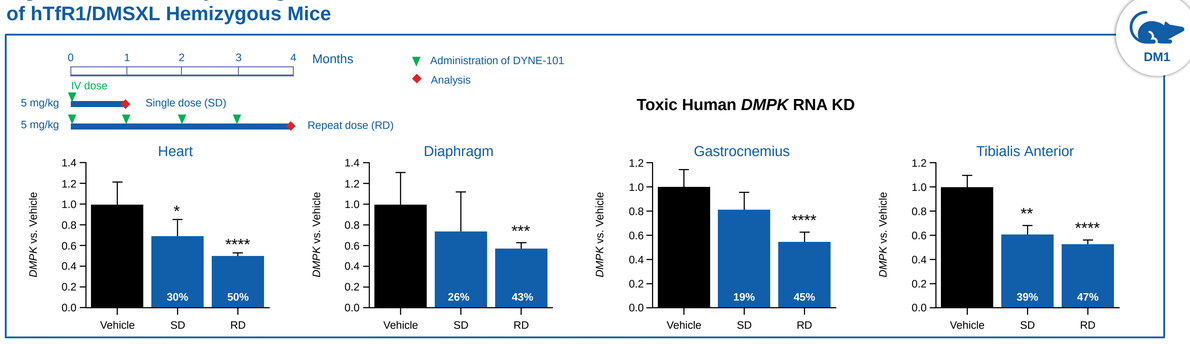
*Based on conclusions of report from third-party CRO. NOAEL, no-observed-adverse-effect level.

### 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



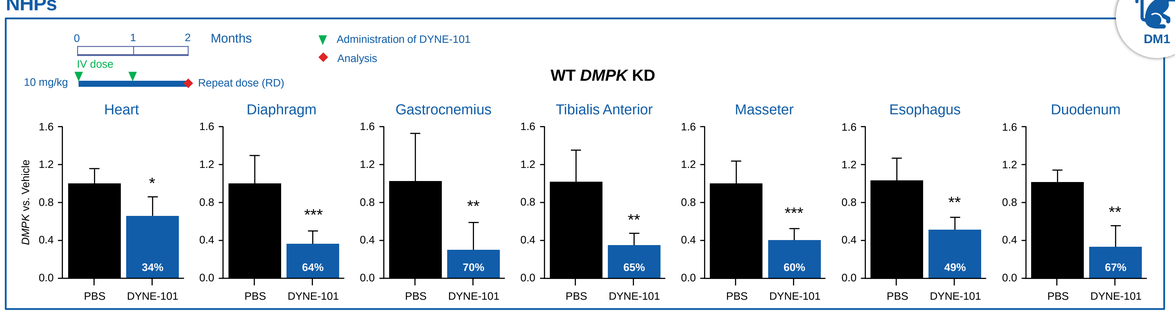

*DMPK* KD measured by qRT-PCR, representative images from *in situ* hybridization chain reaction in heart tissues with quantification on FIJI software, composite splicing index of *Ldb3* exon (E)11, *Mbnl2* exon E6, and *Nfix* E7 mis-splicing measured by qRT-PCR. Data are mean ± SD; n = 7; *$P$ < 0.05 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. Low Monthly Dosing of DYNE-101 Enhances Toxic Human *DMPK* RNA KD in Cardiac and Skeletal Muscles of hTfR1/DMSXL Hemizygous Mice




*DMPK* KD measured by qRT-PCR. Data are means ± standard deviation; n = 4–6 per arm; *$P$ < 0.05; **$P$ < 0.01; ***$P$ < 0.001; ****$P$ < 0.0001, by one-way ANOVA. DMPK, dystrophia myotonica protein kinase; hTfR1, human transferrin receptor 1; IV, intravenous; KD, knockdown; qRT-PCR, real-time quantitative reverse transcription polymerase chain reaction; RD, repeat dose; SD, single dose.

### Figure 9. Repeat Monthly Dosing of DYNE-101 Achieves Significant WT *DMPK* RNA KD in Cardiac and Skeletal Muscles of NHPs




Data are means ± standard deviation; * $P$ < 0.05; **$P$ < 0.01; ***$P$ < 0.001 significant by One-way ANOVA. DMPK, dystrophia myotonica protein kinase; IV, intravenous; KD, knockdown; NHP, non human primate; PBS, phosphate buffered saline; RD, repeat dose; WT, wild type.

## DYNE-101 TOLERABILITY & SAFETY

- DYNE-101 was well-tolerated in a 13-week GLP toxicology study in NHPs*
- No dose limiting toxicity observed up to a maximally feasible dose (dosed once every 3 weeks)
- 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

*Based on conclusions of report from third-party CRO. NOAEL, no-observed-adverse-effect level.

## 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 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, and a 13-week GLP toxicology study demonstrated a favorable safety profile
- The reported data also demonstrate that DYNE-101: reduces toxic human nuclear *DMPK* RNA and foci and corrects splicing defects in the heart of hTfR1/DMSXL mice
- DYNE-101 monthly dosing in hTfR1/DMSXL mice and NHPs achieved significant *DMPK* RNA KD in different muscle types affected by DM1 pathology, and DYNE-101 was well-tolerated in a 13-week GLP toxicology study in NHPs
- The FORCE platform has the potential to provide an effective, re-dosable, and titratable treatment for patients with muscle diseases, including DM1 and DMD.
- The Phase 1/2 DELIVER clinical trial of DYNE-251 for the treatment of DMD and the Phase 1/2 ACHIEVE trial of DYNE-101 for the treatment of DM1 have been initiated.

Data were partially presented at ICNMD 2022 and MDA 2022 and published in Desjardins C, et al. *Nucleic Acids Res*. 2022;gkac641. doi:10.1093/nar/gkac641.

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

**ACKNOWLEDGEMENTS** We thank Dr. Geneviève Gourdon for providing the DMSXL mice and John Hall, Emma O'Donnell, Reshmi Venkatesan, Lydia Schlaefke, and Ryan Russo for their diligent work on these data.

## REFERENCES

1. Daniels TR, et al. *Clin Immunol.* 2006;121:159–176.
2. Barrientos T, et al. *EBioMedicine*. 2015;2:1705–1717.
3. Li Y, et al. *Neural Regen Res*. 2021;16:1308–1316.
4. Kole R, Krieg AM. *Adv Drug Deliv Rev.* 2015;87:104–107.
5. Lim KR, et al. *Drug Des Devel Ther.* 2017;11:533–545.
6. Dowdy SF. *Nat Biotechnol.* 2017;35:222–229.
7. Godfrey C, et al. *EMBO Mol Med.* 2017;9:545–557.
8. Arechavala-Gomeza V, et al. *Curr Gene Ther.* 2012;12:152–160.
9. Thornton CA. *Neurol Clin.* 2014;32:705–719.
10. Thornton CA, et al. *Curr Opin Genet Dev.* 2017;44:135–140.