DYNE-101 TARGETS *DMPK* EXPRESSION TO CORRECT SPLICING IN KEY MUSCLES FOR DM1 PATHOLOGY AND IS WELL TOLERATED IN CYNOMOLGUS MONKEYS

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BACKGROUND

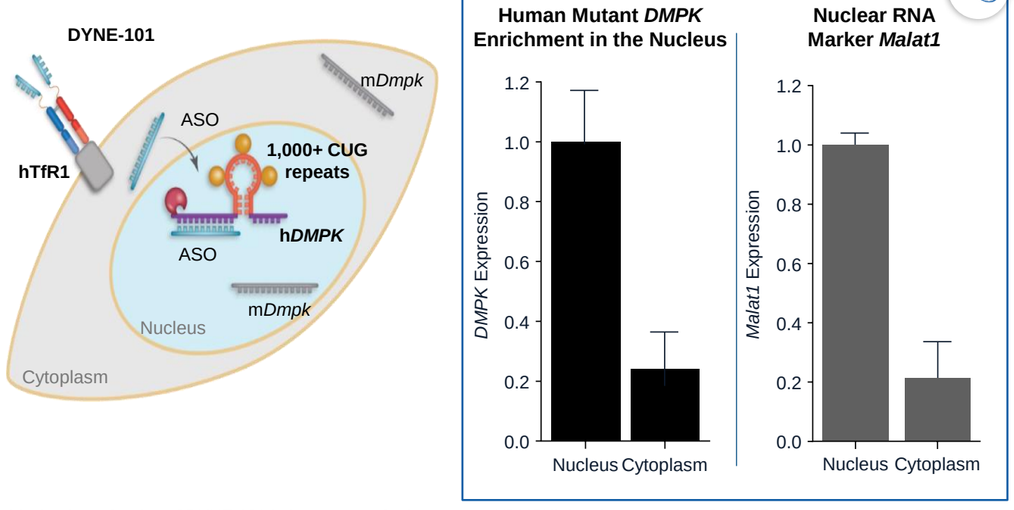
- Myotonic dystrophy Type 1 (DM1) is a rare, debilitating, genetic, progressive neuromuscular disease caused by expansion of CUG repeats in the 3' untranslated region (UTR) of the dystrophia myotonica protein kinase (*DMPK*) RNA[1]
  - *DMPK* transcripts with CUG repeat expansions are trapped in the nucleus and bind to muscleblind-like splicing factors, sequestering them in toxic nuclear foci,[2] ultimately resulting in splicing defects[3]
  - Currently, there are no approved therapies for DM1[4]
- DYNE-101 was designed to target the *DMPK* RNA for RNase-H-mediated degradation by an antisense oligonucleotide (ASO). The ASO is joined by a cleavable valine-citrulline linker to an antigen-binding fragment (Fab) antibody that targets the human transferrin receptor 1 (hTfR1), which is expressed on muscle

METHODS

- Prior to evaluation of DYNE-101 in hTfR1/DMSXL mice, a mouse-specific FORCE conjugate was evaluated in the human skeletal alpha actin (HSA) long repeat (HSA^{LR}) mouse model; HSA^{LR} mice have 250 CTG repeats in the 3' UTR of the *ACTA1* gene, and have a characteristic myotonic phenotype[5]
  - HSA^{LR} mice were administered single intravenous doses of FORCE or an unconjugated ASO targeting *ACTA1* RNA. Analyses were performed on day 14.
- hTfR1/DMSXL mice express the hTfR1 and a human *DMPK* gene with > 1,000 CTG repeats (DMSXL).[2] Hemizygous hTfR1/DMSXL mice exhibit toxic human DMPK trapped in the nuclei of skeletal muscle (gastrocnemius)
- Homozygous hTfR1/DMSXL mice are a novel model that carries two copies of the human *DMPK* gene, yielding higher *DMPK* expression compared with hemizygous DMSXL, and they have a DM1 splicing phenotype
- Fractionation studies were conducted in hTfR1/DMSXL hemizygous mice treated on day 0 with 10 mg/kg DYNE-101 or with phosphate buffered saline (PBS) and analyzed on day 28
  - *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 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 PBS and analyzed on month 1 (SD) or month 4 (RD)
- *DMPK* RNA was assessed in male cynomolgus monkeys treated on month 0 (SD) or treated on months 0 and 1 (RD) with 10 mg/kg DYNE-101 or with PBS and analyzed on month 1 (SD) or month 2 (RD)
- A GLP toxicology study for DYNE-101 was performed in male cynomolgus monkeys

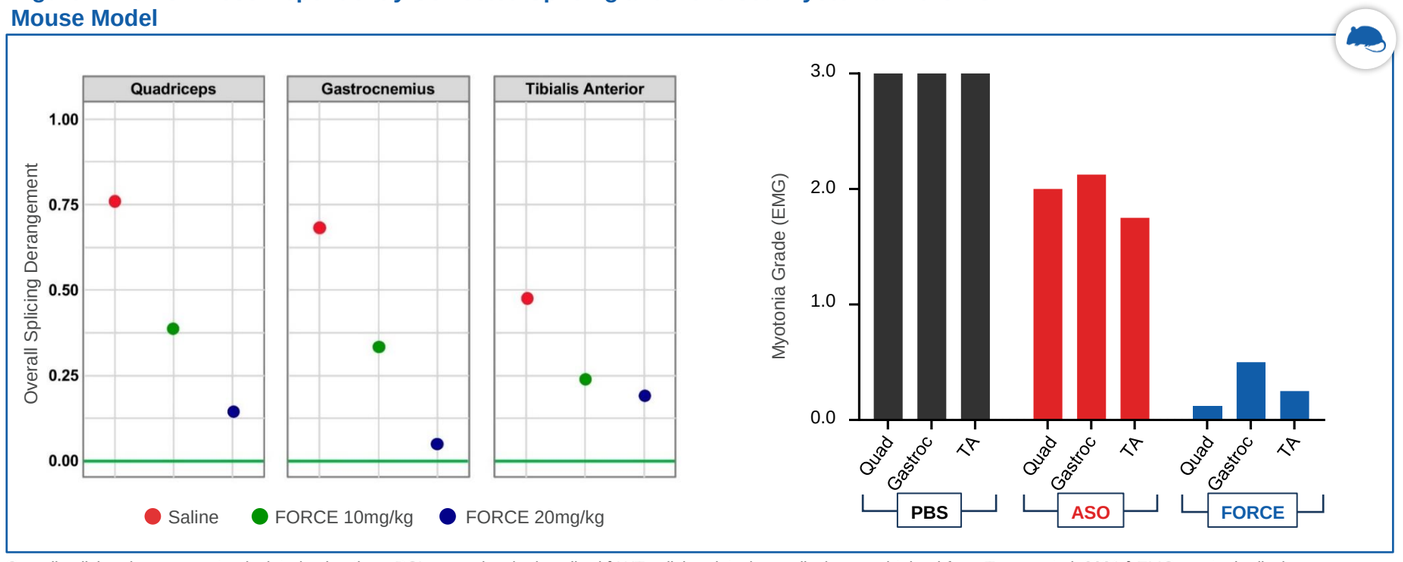
RESULTS

Figure 1. Toxic Human DMPK is Trapped in Nuclei of Skeletal Muscle of hTfR1/DMSXL Hemizygous Mice



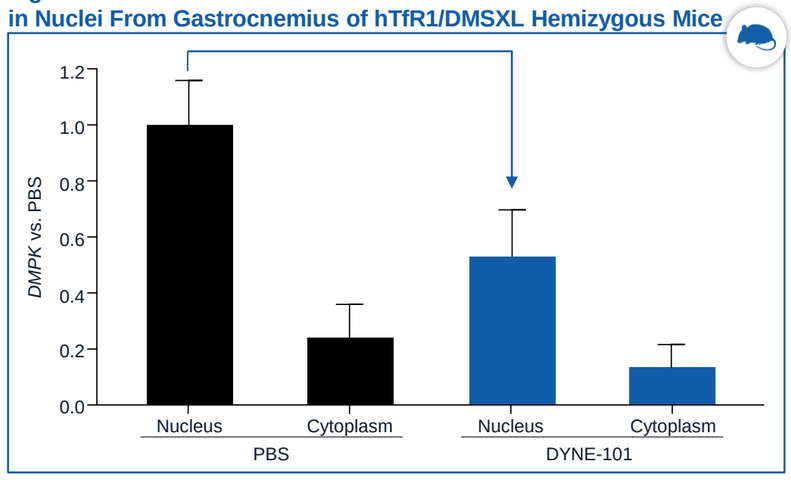
Mechanism of action of DYNE-101 in human transferrin receptor 1/DMSXL hemizygous mice. *DMPK* RNA expression by qRT-PCR in nuclear fractions from hTfR1/DMSXL gastrocnemius confirms nuclear localization. *Malat1* serves as a nuclear RNA marker. Data are mean ± SD; n = 2. ASO, antisense oligonucleotide; Fab, antigen-binding fragment; h*DMPK*, human dystrophia myotonica protein kinase; hTfR1, human transferrin receptor 1; m*Dmpk*, murine dystrophia myotonica protein kinase; qRT-PCR, real-time quantitative reverse transcription polymerase chain reaction; SD, standard deviation.

Figure 2. FORCE Dose-Dependently Corrected Splicing and Reversed Myotonia in the HSA^{LR} DM1 Mouse Model



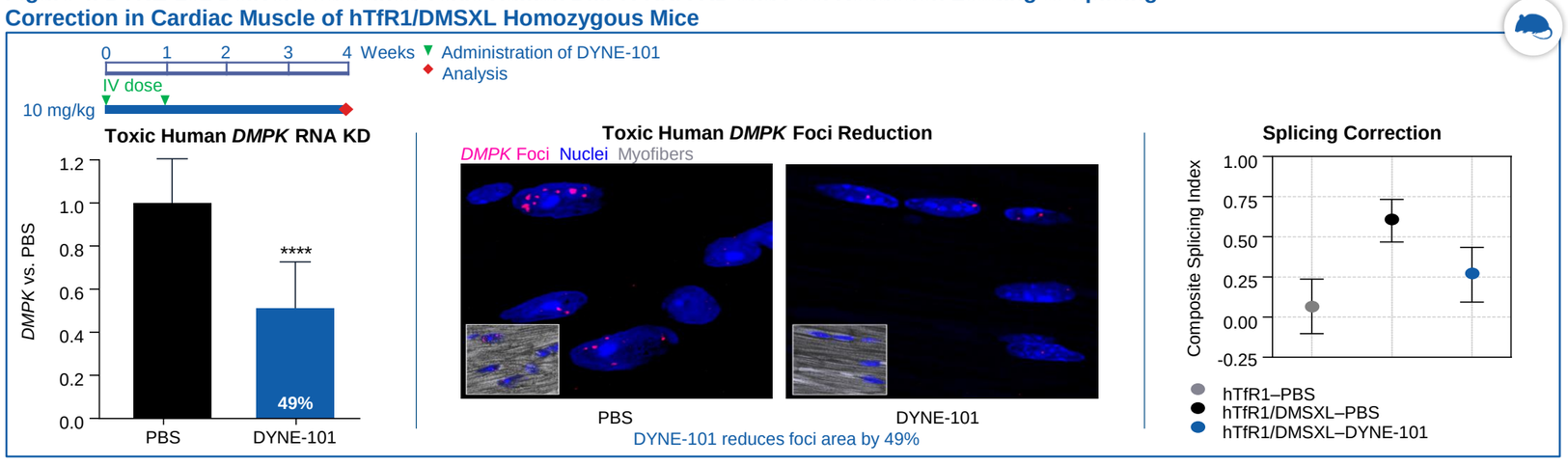
Overall splicing derangement calculated using the mDSI as previously described.[6] WT splicing data (green line) were obtained from Tanner et al. 2021.[6] EMG myotonic discharges were graded by a blinded examiner on a 4-point scale: 0, no myotonia; 1, occasional myotonic discharge in less than 50% of needle insertions; 2, myotonic discharge in greater than 50% of needle insertions; 3, myotonic discharge with nearly every insertion. ASO, antisense oligonucleotide; EMG, electromyography; HSA^{LR}, human skeletal alpha actin long repeat; mDSI, mouse DM splicing index; PBS, phosphate buffered saline; WT, wild type.

Figure 3. DYNE-101 Leads to Robust KD of Toxic Human *DMPK* in Nuclei From Gastrocnemius of hTfR1/DMSXL Hemizygous Mice



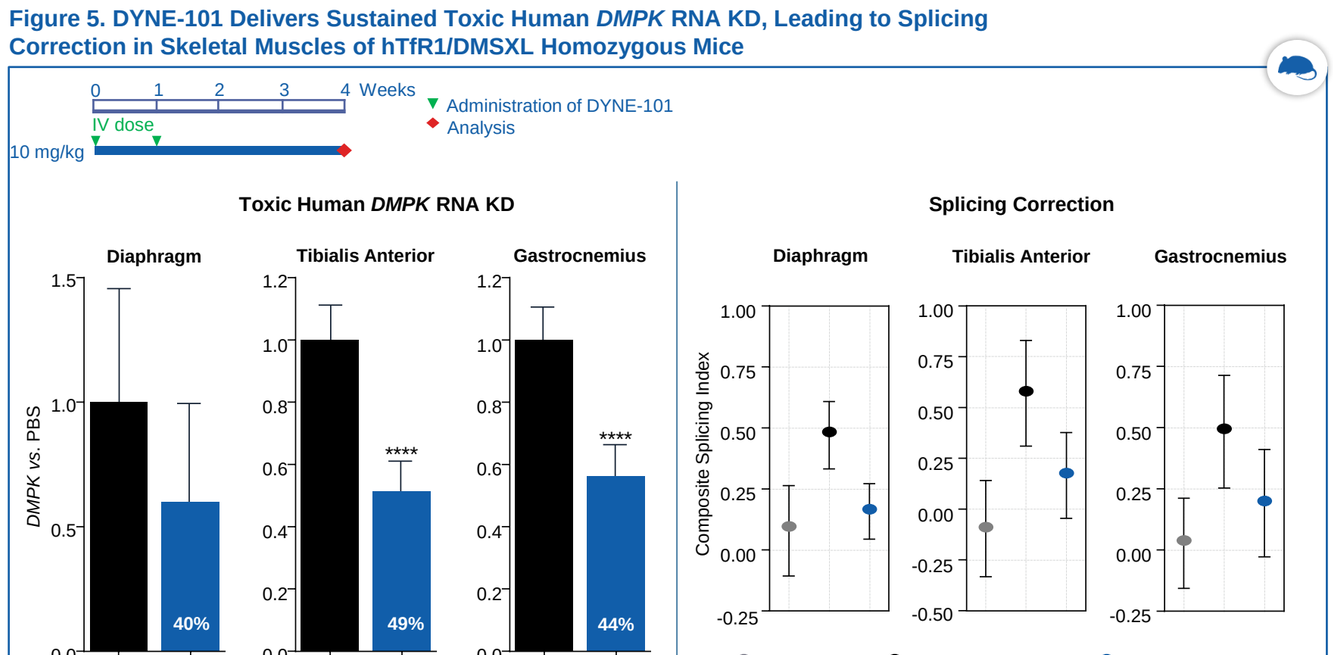
DMPK KD measured by qRT-PCR in nuclear fractions. Data are mean ± SD; n = 2. DMPK, dystrophia myotonica protein kinase; hTfR1, human transferrin receptor 1; KD, knockdown; PBS, phosphate buffered saline; qRT-PCR, real-time quantitative reverse transcription polymerase chain reaction; SD, standard deviation.

Figure 4. DYNE-101 Delivers Sustained Toxic Human *DMPK* RNA KD and Foci Reduction Leading to Splicing Correction in Cardiac Muscle of hTfR1/DMSXL Homozygous Mice



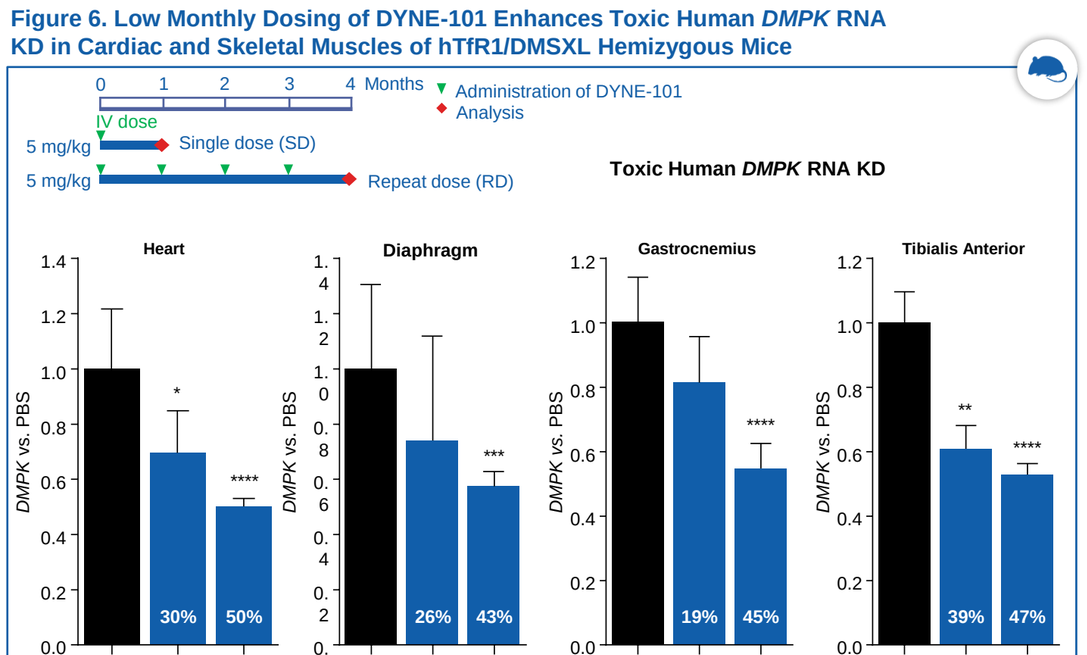
DMPK KD measured by qRT-PCR, representative images from *in situ* hybridization chain reaction in heart tissues with quantification on FIJI software, composite splicing index[5] of *Ldb3* exon (E)11, *Mbnl2* exon E6, and *Nfix* E7 mis-splicing measured by qRT-PCR. Data are mean ± SD; n = 7; **P* < .05; *****P* < .0001, by t-test. DMPK, dystrophia myotonica protein kinase; hTfR1, human transferrin receptor 1; IV, intravenous; KD, knockdown; PBS, phosphate buffered saline; qRT-PCR, real-time quantitative reverse transcription polymerase chain reaction; SD, standard deviation.

Figure 5. 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* < 0.0001, by *t*-test. *DMPK*, dystrophia myotonica protein kinase; hTfR1, human transferrin receptor 1; KD, knockdown; PBS, phosphate buffered saline; qRT-PCR, real-time quantitative reverse transcription polymerase chain reaction; SD, standard deviation.

Figure 6. 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 7. Repeat Monthly Dosing of DYNE-101 Enhances WT *DMPK* KD in Skeletal Muscles of NHPs

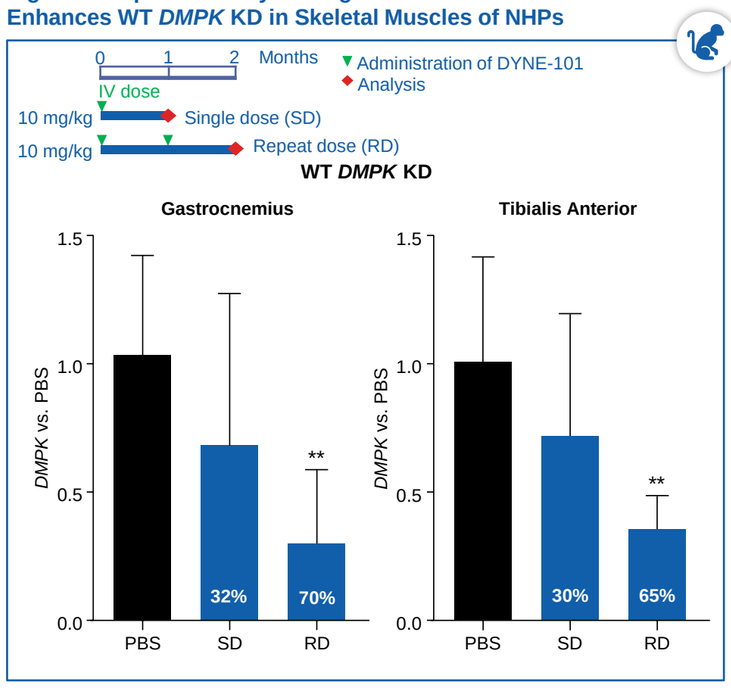
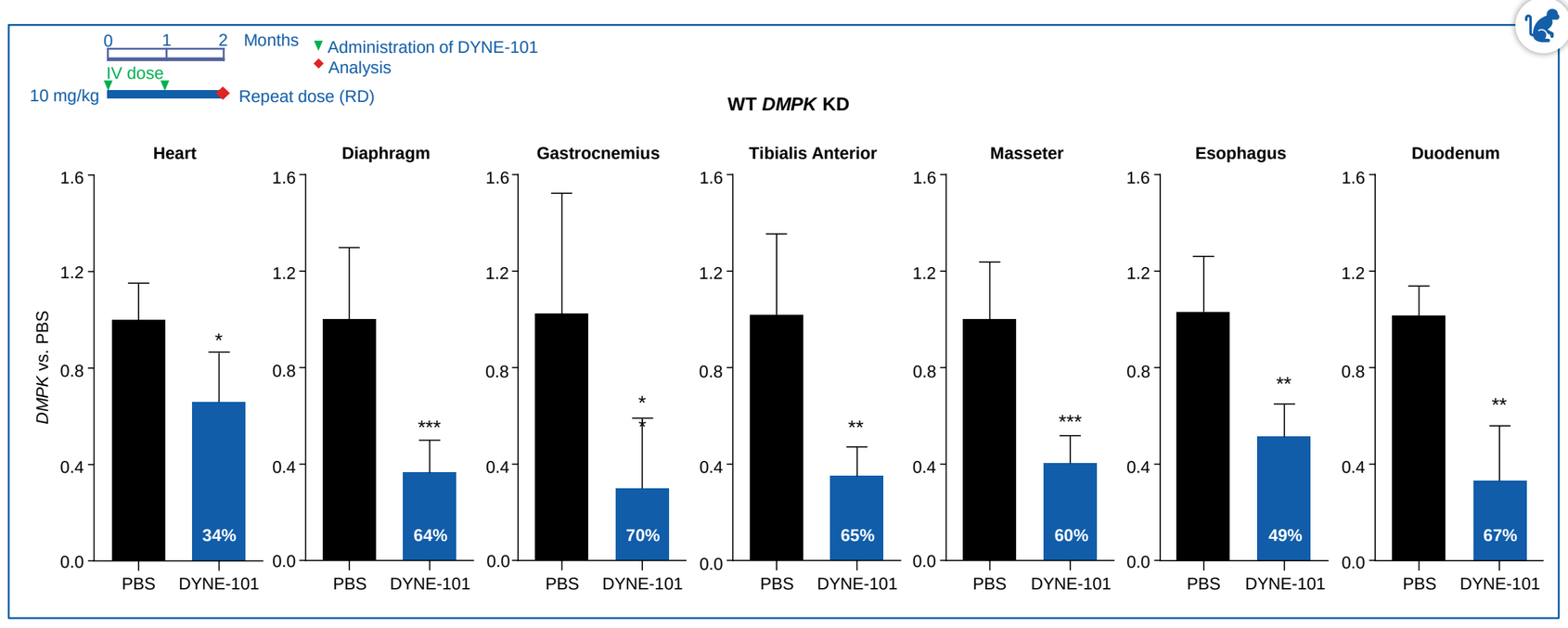


Figure 8. Repeat Monthly Dosing of DYNE-101 Achieves Significant WT *DMPK* KD in Cardiac and Skeletal Muscles of NHPs



DMPK KD measured by qRT-PCR. Data are means ± standard deviation; n = 4–6 per arm. **P* < .05; ***P* < .01; ****P* < .001; by one-way ANOVA. DMPK, dystrophia myotonica protein kinase; IV, intravenous; KD, knockdown; NHP, non human primate; PBS, phosphate buffered saline; RD, repeat dose; qRT-PCR, real-time quantitative reverse transcription polymerase chain reaction; SD, single dose; WT, wild type.

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. NHP, non human primate; NOAEL, No-Observed-Adverse-Effect-Level.

CONCLUSIONS

- In the HSA^{LR} mouse model, FORCE demonstrated correction of spliceopathy and improved myotonia to a greater extent than unconjugated ASO
- DYNE-101 demonstrated ability to target toxic human *DMPK* RNA in the nucleus and correct splicing in cardiac and skeletal muscle of hTfR1/DMSXL mice, as well as reduce *DMPK* foci in the heart
- DYNE-101 low monthly dosing in hTfR1/DMSXL mice and NHPs achieved significant *DMPK* RNA knockdown in different muscle types affected by DM1 pathology
- DYNE-101 was well-tolerated in a 13-week GLP toxicology study in NHPs
- These data support initiation of the Phase 1/2 ACHIEVE clinical trial of DYNE-101 for the treatment of DM1

All data were previously presented at the 2022 ASGCT Annual Meeting (Zanotti et al. P.17) , 2022 ICNMD Congress (Picariello et al. eP02.06.01), and January 2022 Dyne Corporate Presentation.

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DISCLOSURE INFORMATION **CT**, advisory board (Dyne Therapeutics Inc.); **ZT**, no competing interests; all other authors are employees of Dyne Therapeutics Inc. and may hold Dyne stock and/or stock options.

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