DYNE-101 CORRECTS THE DM1 SPLICING PHENOTYPE OF hTfR1/DMSXL MICE AND IS WELL TOLERATED IN NHP

Tyler Picariello, Ryan Russo, Lydia Schlaefke, Scott Hildebrand, John Najim, Qifeng Qiu, Timothy Weeden, John W. Davis II, Stefano Zanotti, Oxana Beskrovnaya

Dyne Therapeutics Inc., Waltham, MA, USA

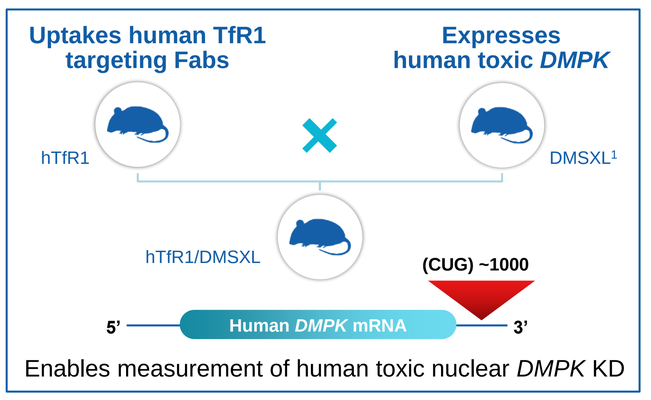
BACKGROUND

- Myotonic dystrophy type 1 (DM1) is a rare, debilitating, genetic, progressive neuromuscular disease caused by expansion of CUG repeats in the 3' untranslated region of the dystrophia myotonica protein kinase (*DMPK*) RNA[1]
  - *DMPK* transcripts with CUG repeats expansion are trapped in the nucleus and bind to muscleblind-like (MBNL) 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 clinically validated valine-citrulline linker to an antigen-binding fragment (Fab) antibody that targets the human transferrin receptor 1 (hTfR1), which is highly expressed on muscle.

METHODS

- hTfR1/DMSXL mice express the hTfR1 and a human *DMPK* gene with > 1,000 CTG repeats (DMSXL)[2]
- 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 vehicle 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

Figure 1. hTfR1/DMSXL mice are a PK/PD model

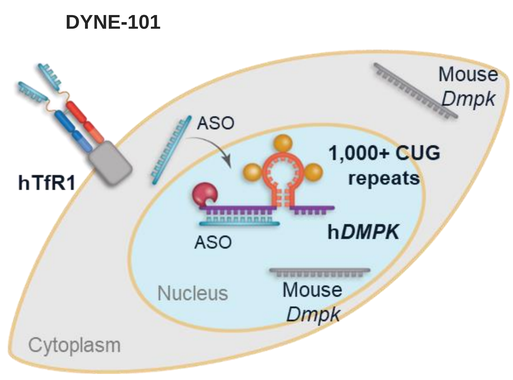


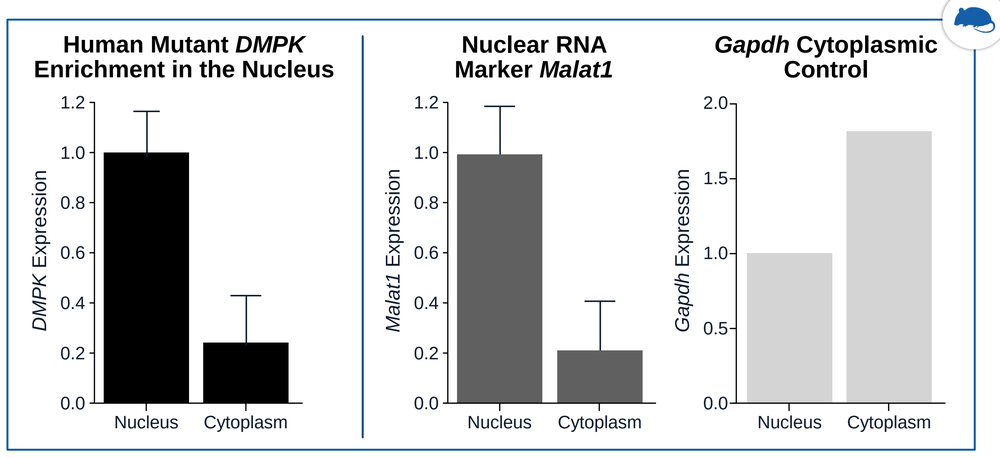
Enables measurement of human toxic nuclear *DMPK* KD

DMPK, dystrophia myotonica protein kinase; Fabs, fragment antigen-binding; hTfR1, human transferrin receptor 1; KD, knockdown; PD, pharmacodynamics; PK, pharmacokinetics.

RESULTS

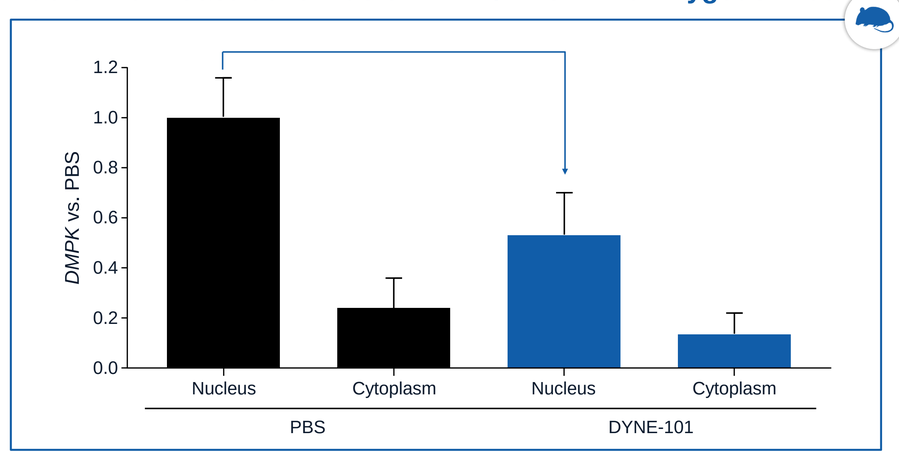
Figure 2. Toxic Human *DMPK* is Trapped in Nuclei of Skeletal Muscle of hTfR1/DMSXL Hemizygous Mice





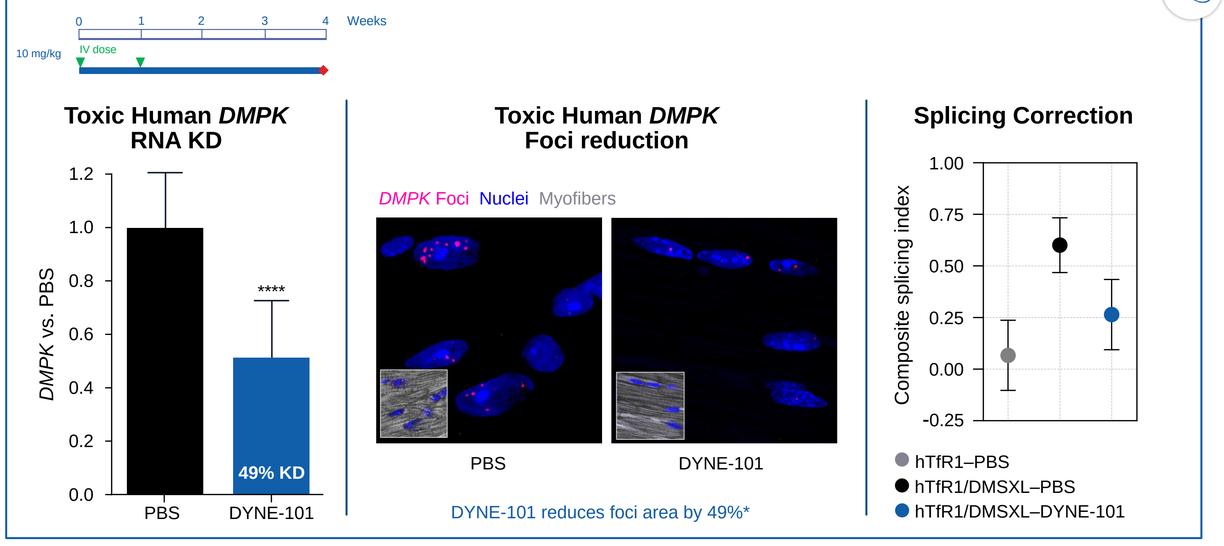
DMPK RNA expression by qRT-PCR in nuclear fractions from hTfR1/DMSXL gastrocnemius confirms nuclear localization. *Malat1* serves as a nuclear RNA marker. Data are mean ± SD; n = 2. ASO, antisense oligonucleotide; *DMPK*, dystrophia myotonica protein kinase; hTfR1, human transferrin receptor 1; qRT-PCR, real-time quantitative reverse transcription polymerase chain reaction; SD, standard deviation.

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



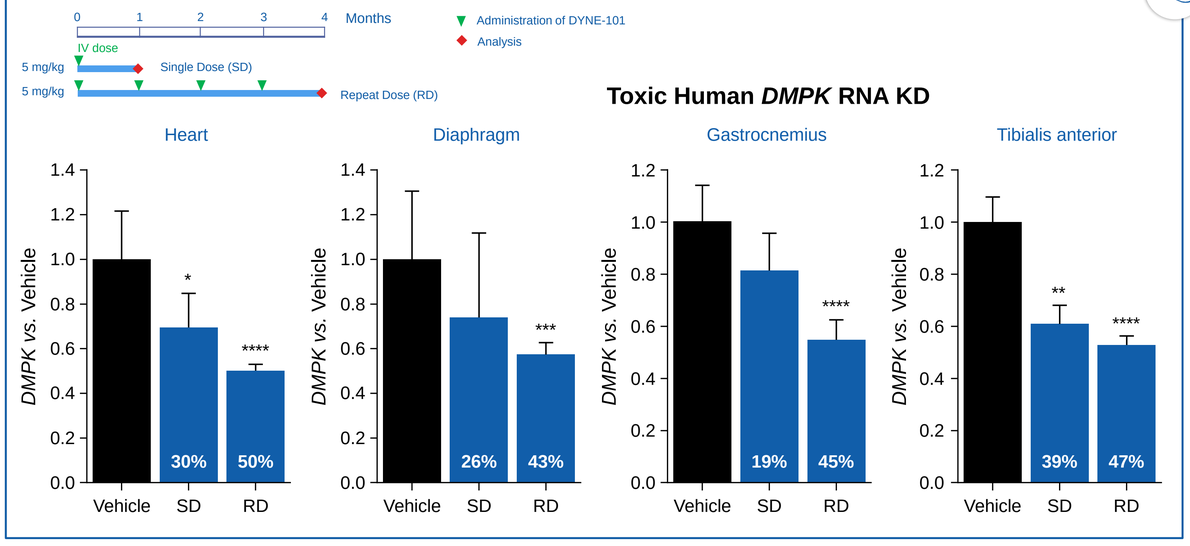
DMPK KD measured by qRT-PCR in nuclear fractions. Data are mean ± SD; n = 2. *DMPK*, dystrophia myotonica protein kinase; hTfR1, human transferrin receptor 1; KD, knockdown; PBS, phosphate buffered saline; qRT-PCR, real-time quantitative reverse transcription polymerase chain reaction; SD, standard deviation.

Figure 4. DYNE-101 Delivers Sustained Toxic Human *DMPK* RNA KD and Foci Reduction Leading to Splicing Correction in the Heart 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; KD, knockdown; PBS, phosphate buffered saline; qRT-PCR, real-time quantitative reverse transcription polymerase chain reaction; SD, standard deviation.

Figure 5. 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, 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 means ± SD; n = 4 – 6 per arm; *P < .05; **p< .01; ***p< .001, ****p< .0001, by One-way ANOVA

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

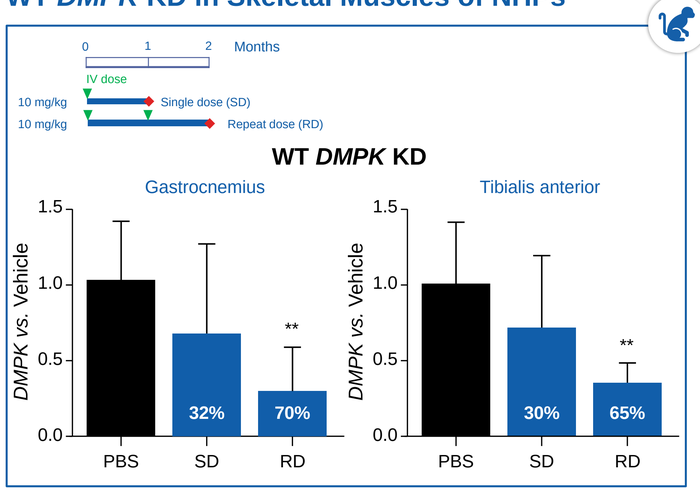
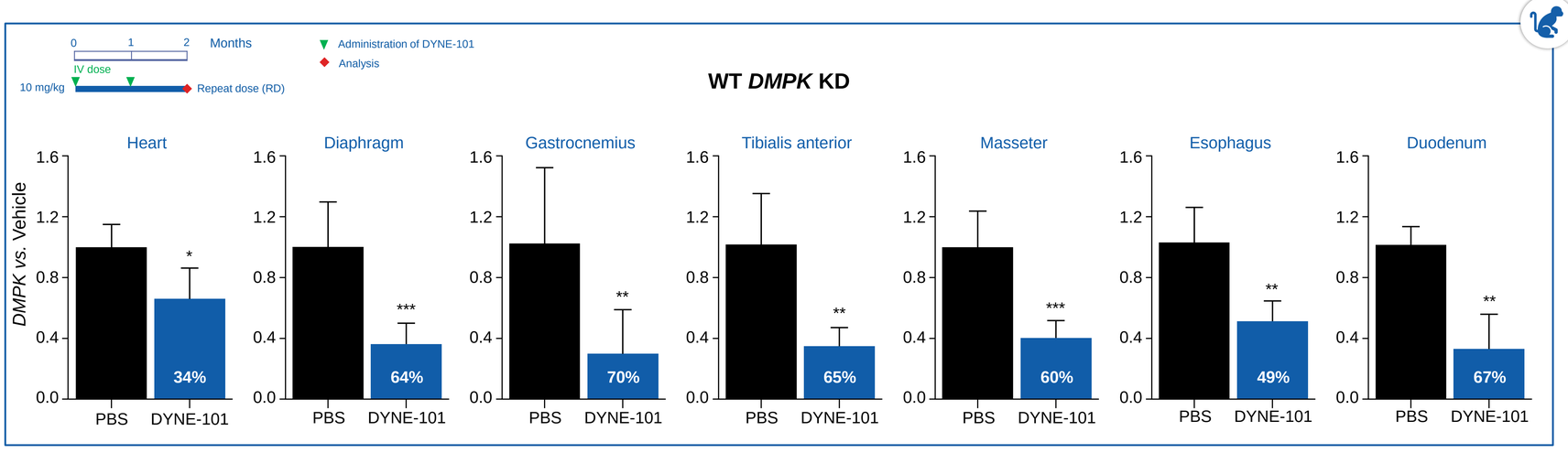


Figure 7. Repeat Monthly Dosing of DYNE-101 Achieves Significant WT *DMPK* KD in Cardiac and Skeletal Muscles of NHPs



DMPK KD measured by qRT-PCR. Data are means ± SD; n = 4 – 6 per arm; *P < .05; **p< .01; ***p< .001, ****p< .0001, by One-way ANOVA. *DMPK*, dystrophia myotonica protein kinase; KD, knockdown; RD, repeat dose; qRT-PCR, real-time quantitative reverse transcription polymerase chain reaction; SD, single dose; sd, standard deviation; 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 (No-Observable-Adverse-Effect-Level) was identified at the highest dose tested

*Based on conclusions of report from third-party CRO.

CONCLUSIONS

- DYNE-101 demonstrated ability to target toxic human *DMPK* RNA in the nucleus, reduce *DMPK* foci, and correct splicing in the hTfR1/DMSXL model of DM1
- DYNE-101 monthly dosing in hTfR1/DMSXL mice and NHPs achieved significant *DMPK* RNA KD 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 advancement of DYNE-101 into the clinic for the treatment of DM1

REFERENCES

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

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