DYNE-101 ACHIEVES DURABLE KNOCKDOWN OF TOXIC HUMAN NUCLEAR *DMPK* RNA AND CORRECTION OF SPLICING IN THE hTfR1/DMSXL MOUSE MODEL OF DM1

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BACKGROUND

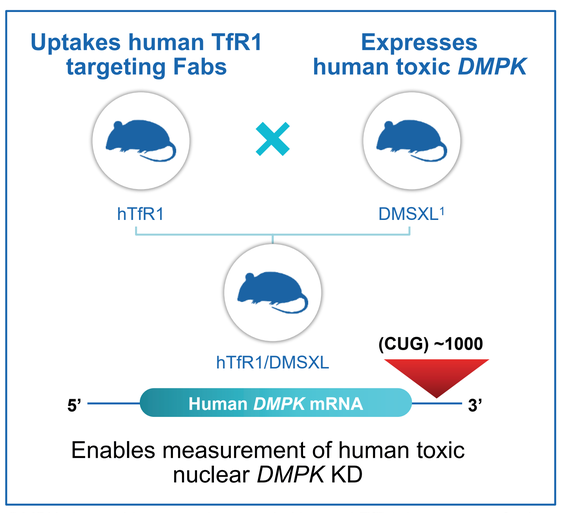
- Myotonic dystrophy type 1 (DM1) is a rare, debilitating, genetic, progressive neuromuscular disease caused by expansion of CUG repeats in the 3' untranslated region of the dystrophia myotonica protein kinase (*DMPK*) RNA[1]
  - *DMPK* transcripts with CUG repeats expansion are trapped in the nucleus 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
- Using an innovative DM1 mouse model (hTfR1/DMSXL hemizygotes), we report:
  - Direct *in vivo* evidence that human mutant *DMPK* RNA is trapped in the nucleus of hTfR1/DMSXL muscle
  - DYNE-101 acts in the nucleus to degrade human toxic *DMPK* RNA
- Using hTfR1/DMSXL homozygous mice that develop spliceopathy, we demonstrate that DYNE-101:
  - Effectively reduces human toxic *DMPK* knockdown (KD), degrades foci, and corrects spliceopathy in the heart
  - Corrects splicing defects across multiple skeletal muscles
- DYNE-101 was well tolerated in a 13-week GLP toxicology study in non-human primates (NHPs)

METHODS

- hTfR1/DMSXL mice express the hTfR1 and a human *DMPK* gene with > 1000 CTG repeats (DMSXL)[2] that is representative of a severe DM1 phenotype
- 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 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
- A GLP toxicology study for DYNE-101 was performed in male Asian cynomolgus monkeys

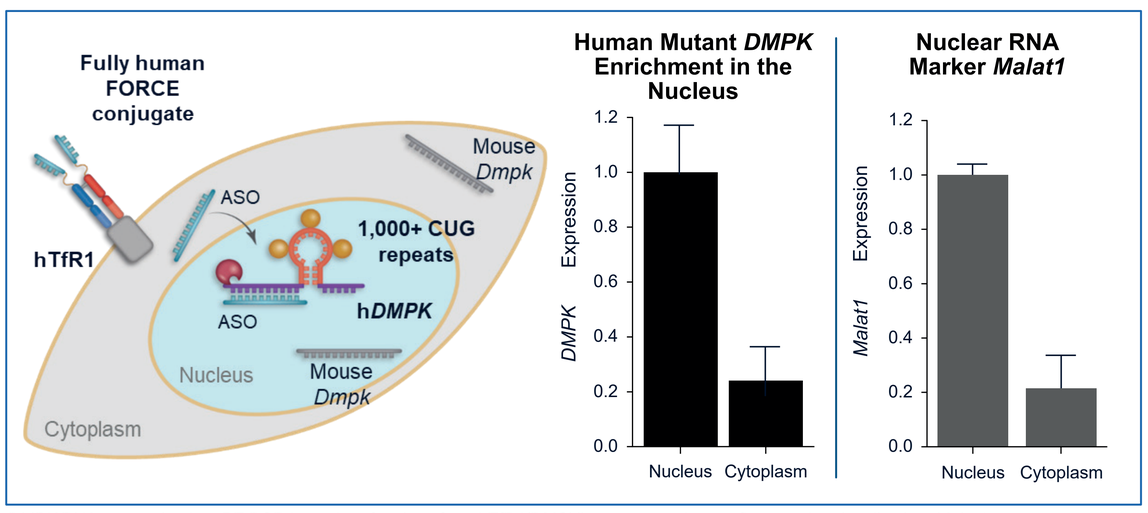
RESULTS

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



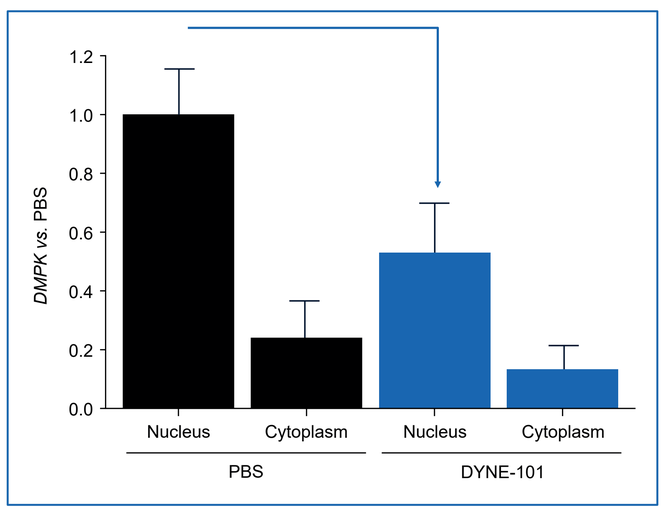
DMPK, dystrophia myotonica protein kinase; hTfR1, human transferrin receptor 1; KD, knockdown; PD, pharmacodynamics; PK, pharmacokinetics.

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



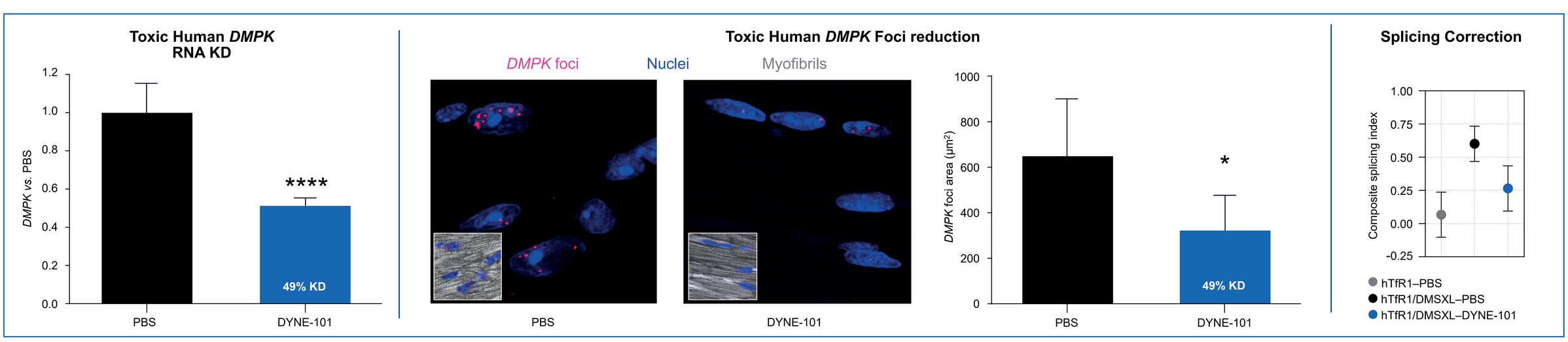
DMPK RNA expression by qRT-PCR in nuclear fractions from hTfR1/DMSXL gastrocnemius confirms nuclear localization. *Malat1* serves as a nuclear RNA marker. Data are mean ± SD; n = 2. ASO, antisense oligonucleotide; *DMPK*, dystrophia myotonica protein kinase; hTfR1, human transferrin receptor 1; qRT-PCR,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



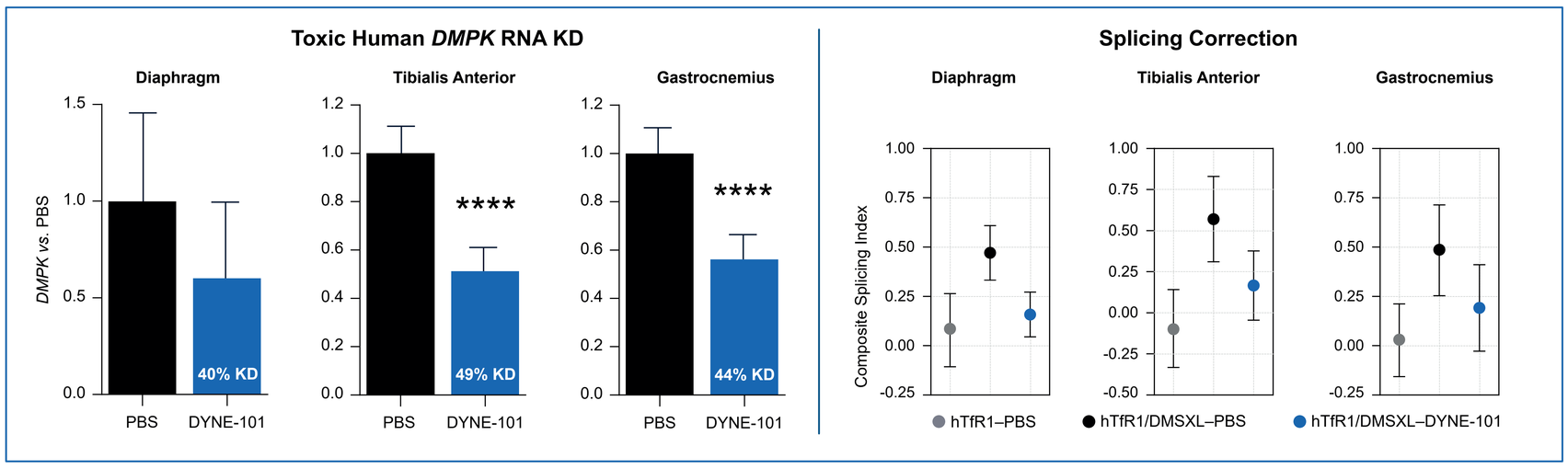
DMPK KD measured by qRT-PCR in nuclear fractions. Data are mean ± SD; n = 2. *DMPK*, dystrophia myotonica protein kinase; hTfR1, human transferrin receptor 1; KD, knockdown; PBS, phosphate buffered saline; qRT-PCR, real-time quantitative reverse transcription polymerase chain reaction; SD, standard deviation.

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. 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, real-time quantitative reverse transcription polymerase chain reaction; SD, standard deviation.

DYNE-101 was well tolerated in a 13-week GLP toxicology study[1]

- 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

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

CONCLUSIONS

- These data demonstrate that DYNE-101:
  - Reduces toxic human nuclear *DMPK* RNA and foci and corrects splicing defects in the hTfR1/DMSXL model of DM1
  - Is well tolerated in a 13-week GLP tox study in NHPs
  - These results support further development of DYNE-101, including a planned clinical study

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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