THE FORCE™ PLATFORM ACHIEVES ROBUST KNOCKDOWN OF TOXIC HUMAN NUCLEAR *DMPK* RNA AND FOCI REDUCTION IN DM1 CELLS AND IN NEWLY DEVELOPED hTfR1/DMSXL MOUSE MODEL

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BACKGROUND

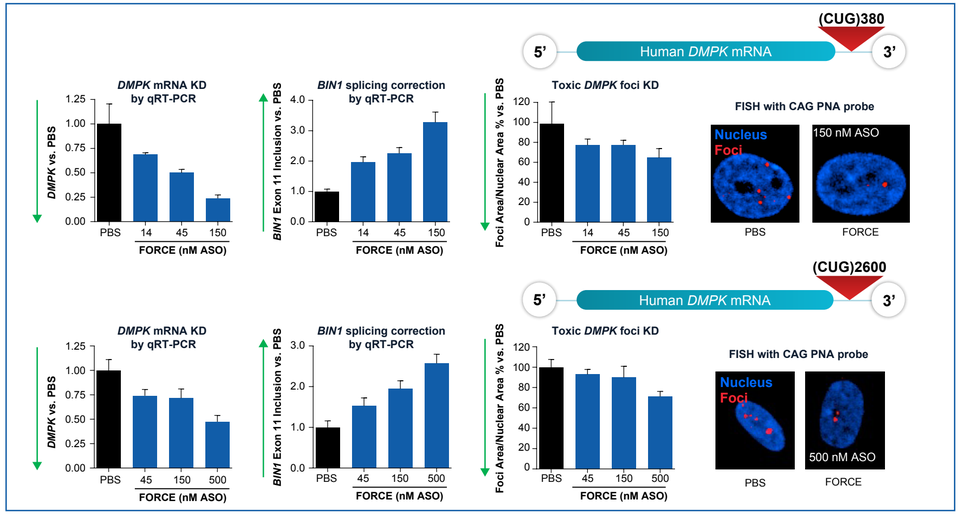
- Myotonic dystrophy type 1 (DM1) is a rare, debilitating, genetic, progressive neuromuscular disease caused by expansion of CUG repeats in the 3' untranslated region of the dystrophia myotonica protein kinase (*DMPK*) RNA[1]
  - *DMPK* transcripts with CUG repeats expansion are trapped in the nucleus 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]
- To address the genetic basis of DM1, we designed a FORCE conjugate 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 DM1 patient-derived cells we report that a fully human FORCE conjugate:
  - Leads to *DMPK* knockdown (KD)
  - Corrects *BIN1* splicing
  - Reduces toxic *DMPK* nuclear foci
- In an innovative *in vivo* DM1 model developed by Dyne (hTfR1/DMSXL mice), we demonstrated that our FORCE conjugate:
  - Targets the genetic driver of DM1, namely human toxic *DMPK*, in cardiac and skeletal muscle
  - Reduces toxic human *DMPK* foci in the heart
  - Leads to substantial toxic human *DMPK* KD with low and infrequent dosing

METHODS

- DM1 patient myotubes containing short (380) and long (2600) CUG repeats in the *DMPK* RNA were used for *in vitro* analyses
- *DMPK* RNA KD and foci reduction were assessed in a novel hTfR1/ DMSXL mouse model that expresses hTfR1 and human *DMPK* RNA containing > 1000 CUG repeats, representative of a severe DM1 phenotype (DMSXL mouse described previously[2])

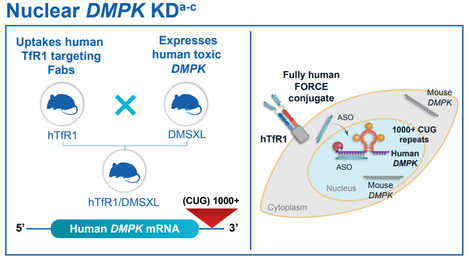
RESULTS

Figure 1. FORCE Conjugate Demonstrated Dose-dependent *DMPK* KD, Splicing Correction, and Foci Reduction in DM1 Myotubes with 380 and 2600 CTG Repeats



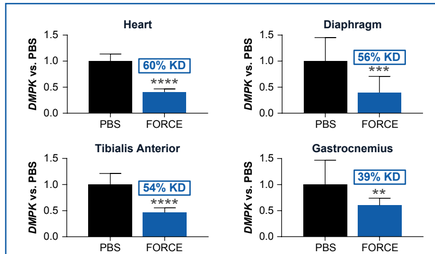
Data are mean ± SD; n = 4. ASO, antisense oligonucleotide; *DMPK*, dystrophia myotonica protein kinase; DM1, myotonic dystrophy type 1; FISH, fluorescence *in situ* hybridization; KD, knockdown; PBS, phosphate-buffered saline; PNA, peptide nucleic acid; qRT-PCR, quantitative reverse-transcriptase polymerase chain reaction.

Figure 2. The hTfR1/DMSXL Innovative Mouse Model Evaluated FORCE Conjugate Pharmacodynamics by Measuring Toxic Human Nuclear *DMPK* KD[a-c]



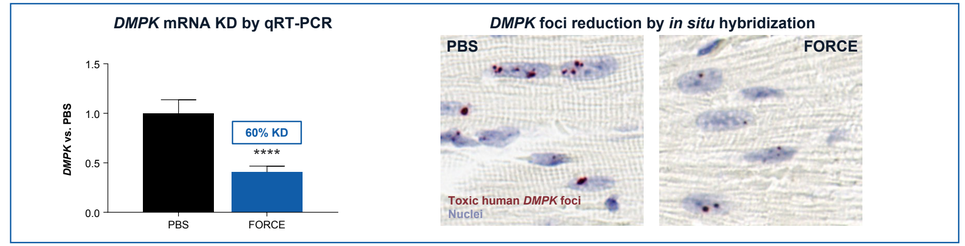
[a]DMSXL mice were first described in *PLoS Genetics* 2012, 8(11):e1003043.
[b]Expresses human TfR1 receptor, enabling use of human TfR1-targeting Fabs.
[c]Underestimates potency, expressing >10 times less human toxic *DMPK* vs mouse *DMPK*. ASO, antisense oligonucleotide; *DMPK*, dystrophia myotonica protein kinase; Fabs, antigen-binding fragments; KD, knockdown; TfR1, transferrin receptor 1.

Figure 3. Repeat Dose of FORCE Conjugate Demonstrated Robust Toxic Human *DMPK* KD in hTfR1/DMSXL Mice after 14 Days



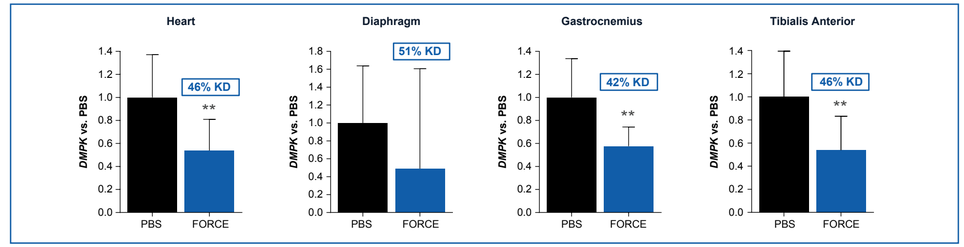
2 × 10 mg/kg, day 0 and day 7, analyzed day 14; Data are mean ± SD; **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001, significant by *t* test.
DMPK, dystrophia myotonica protein kinase; KD, knockdown; PBS, phosphate-buffered saline.

Figure 4. FORCE Conjugate Demonstrated Robust Toxic Human *DMPK* mRNA and Foci Reduction in the Heart of hTfR1/DMSXL Mice



10 mg/kg ASO day 0 and day 7, analyzed day 14; data are mean ± SD; n = 6–9; *****P* < 0.0001, significant by *t* test.
ASO, antisense oligonucleotide; *DMPK*, dystrophia myotonica protein kinase; KD, knockdown; qRT-PCR, quantitative reverse-transcriptase polymerase chain reaction.

Figure 5. Single Dose of FORCE Conjugate Achieved Sustained Toxic Human *DMPK* KD at Week 4 in hTfR1/DMSXL Mice



10 mg/kg ASO day 0, analyzed day 28; data are mean ± SD; n = 6; ***P* < 0.01, significant by *t* test.
ASO, antisense oligonucleotide; *DMPK*, dystrophia myotonica protein kinase; KD, knockdown; PBS, phosphate-buffered saline.

CONCLUSIONS

- We designed a FORCE conjugate to address the genetic basis of DM1 by targeting toxic nuclear *DMPK* RNA
- These data demonstrated that our FORCE conjugate can:
  - Correct the DM1 phenotype of patient-derived myoblast cultures with a range of repeats, including those representative of severe DM1
  - Reduce toxic nuclear human *DMPK* foci in cardiac muscle of hTfR1/DMSXL mice
  - Lead to sustained KD of toxic nuclear human *DMPK* in hTfR1/DMSXL mice after a single dose
- These data strongly support further development of our FORCE conjugate, including a planned clinical study

REFERENCES

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2. Huguet A, et al. *PLoS Genet*. 2012;8:e1003043. doi:10.1371/journal.pgen.1003043
3. Nakamori M, et al. *Ann Neurol*. 2013;74:862–872.
4. Thornton CA, et al. *Curr Opin Genet Dev*. 2017;44:135–140.

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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