Identification and Characterization of Modified Antisense Oligonucleotides Targeting *DMPK* in Mice and Nonhuman Primates for the Treatment of Myotonic Dystrophy Type 1[§]

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ABSTRACT

Myotonic dystrophy type 1 (DM1) is the most common form of muscular dystrophy in adults. DM1 is caused by an expanded CTG repeat in the 3'-untranslated region of *DMPK*, the gene encoding dystrophin myotonia protein kinase (DMPK). Antisense oligonucleotides (ASOs) containing 2',4'-constrained ethyl-modified (cEt) residues exhibit a significantly increased RNA binding affinity and in vivo potency relative to those modified with other 2'-chemistries, which we speculated could translate to enhanced activity in extrahepatic tissues, such as muscle. Here, we describe the design and characterization of a cEt gapmer DMPK ASO (ISIS 486178), with potent activity in vitro and in vivo against mouse, monkey, and human *DMPK*. Systemic delivery of unformulated ISIS 486178 to wild-type mice decreased *DMPK*

mRNA levels by up to 90% in liver and skeletal muscle. Similarly, treatment of either human *DMPK* transgenic mice or cynomolgus monkeys with ISIS 486178 led to up to 70% inhibition of *DMPK* in multiple skeletal muscles and ~50% in cardiac muscle in both species. Importantly, inhibition of *DMPK* was well tolerated and was not associated with any skeletal muscle or cardiac toxicity. Also interesting was the demonstration that the inhibition of *DMPK* mRNA levels in muscle was maintained for up to 16 and 13 weeks post-treatment in mice and monkeys, respectively. These results demonstrate that cEt-modified ASOs show potent activity in skeletal muscle, and that this attractive therapeutic approach warrants further clinical investigation to inhibit the gain-of-function toxic RNA underlying the pathogenesis of DM1.

Introduction

The genetic basis of myotonic dystrophy type 1 (DM1) is a CTG repeat expansion in the 3'-untranslated region (UTR) of the gene encoding dystrophin myotonia protein kinase (DMPK) (Brook et al., 1992). Transcription of the *DMPK*-CTG^{exp} gene produces an RNA, *DMPK*-CUG^{exp}, which contains a highly structured 3'-UTR region (Mooers et al., 2005). This repeat-containing RNA is retained in the nucleus and binds to multiple copies of splicing factors, such as muscleblind-like 1 protein (MBLN1), limiting their availability to regulate alternative splicing of several important muscle-expressed genes (Davis et al., 1997; Philips et al., 1998; Jiang et al., 2004). The resulting misregulated splicing or "spliceopathy"

likely underlies several of the symptoms of DM1, including myotonia (delayed relaxation of muscle due to repetitive action potential) and insulin resistance and possibly muscle weakness and wasting (Philips et al., 1998; Savkur et al., 2001; Charlet-B et al., 2002; Mankodi et al., 2002; Jiang et al., 2004). Additional clinical symptoms of DM1 include cataracts, gastrointestinal abnormalities, hypersomnia, and cardiac conduction defects (Udd and Krahe, 2012). Similar gain-of-function mechanisms by repetitive RNA have recently been proposed in myotonic dystrophy type 2 (Liquori et al., 2001), Fuch's endothelial corneal dystrophy (Du et al., 2015), familial amyotrophic lateral sclerosis, and several forms of hereditary ataxia.

Currently, there are no therapies that alter the course of DM1. This is due, in large part, to the inability of the most commonly employed therapeutic modalities to effectively target a disease-causing toxic RNA. Therapeutic nucleic acid approaches, such as antisense technology, have significant potential to target disease-associated RNA molecules as antisense oligonucleotides (ASOs) can be rationally designed

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ABBREVIATIONS: AR, androgen receptor; ASO, antisense oligonucleotide; cEt, 2',4'-constrained ethyl; DM1, myotonic dystrophy type 1; DMPK, dystrophin myotonia protein kinase; ECG, electrocardiogram; FBS, fetal bovine serum; hDMPK, human *DMPK*; hSKMC, human skeletal muscle cell; MBLN1, muscleblind-like 1 protein; mDMPK, mouse *DMPK*; MOE, 2'-(2-methoxyethyl)-D-ribose; PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction; TA, tibialis anterior; UTR, untranslated region; WT, wild type.

based solely on gene sequence information (Bennett and Swayze, 2010). This technology has advanced in recent years, and positive clinical trial data of unformulated ASOs delivered systemically has been reported in several disease areas (van Deutekom et al., 2007; Raal et al., 2010; Goemans et al., 2011; Saad et al., 2011; Gaudet et al., 2014; Büller et al., 2015). Importantly, the systemically administered ASO, mipomersen sodium, which targets the apolipoprotein B transcript, has gained U.S. Food and Drug Administration approval as a treatment of homozygous familial hypercholesterolemia (Lee et al., 2013). Second-generation ASOs have chemically modified 2'-*O*-methoxyethyl (MOE) residues and a phosphorothioate backbone. Although 2'-MOE ASOs primarily distribute to hepatic and renal tissues, a recent study demonstrated that a 2'-MOE-modified ASO designed to target nuclear-retained noncoding RNAs had unexpectedly potent activity in multiple skeletal muscles in the *HSA*^{LR} mouse model of DM1 (Wheeler et al., 2012). *HSA*^{LR} mice exhibit many features of DM1 myopathology, including nuclear retention of the toxic CUG^{exp} RNA, spliceopathy, and myotonia (Mankodi et al., 2000). Systemic treatment of these mice with the 2'-MOE ASO reduced the levels of the disease-causing toxic CUG^{exp} RNA, normalized splicing, and completely reversed myotonia, and interestingly, ASO treatments had greater activity on the nuclear-retained transcript (CUG^{exp} RNA) than the nonnuclear-retained transcript (CUG^{non exp} RNA) (Wheeler et al., 2012). Taken together, these data demonstrate the potential of the ASO approach to therapeutically target nuclear-retained toxic CUG^{exp} RNA underlying DM1.

Recently, a novel high-affinity class of ASOs that contain 2'-4'-constrained ethyl (cEt) modifications has been described (Seth et al., 2009). The cEt ASOs have significantly enhanced in vivo potency compared with 2'-MOE-modified ASOs and a favorable safety profile (Seth et al., 2009; Burel et al., 2013). Here, we describe the identification and in vitro and in vivo characterization of ISIS 486178, a cEt ASO that targets mouse, monkey, and human *DMPK* mRNA. When administered by s.c. injection, the cEt-modified *DMPK* ASO has potent activity against *DMPK* in skeletal and cardiac muscle in normal mice, human *DMPK* transgenic mice, and cynomolgus monkeys.

Material and Methods

Cell Culture. Human skeletal muscle cells (hSKMCs) were obtained from ScienCell Research Laboratories (Carlsbad, CA) and grown in skeletal muscle cell medium. HepG2 cells were obtained from American Type Culture Collection (Manassas, VA) and grown in minimum Eagle's medium containing 10% fetal bovine serum (FBS) supplemented with nonessential amino acids and sodium pyruvate (Life Technologies, Thermo Fisher Scientific, Carlsbad, CA). Screening results were confirmed in muscle cells from DM1 patients, which were maintained in Ham's F-10 Nutrient Mixture (Life Technologies) supplemented with 20% heat-inactivated FBS, 1% penicillin-streptomycin, and 2.5 ng/ml recombinant human fibroblast growth factor. Lead candidate *DMPK*-targeting ASOs were evaluated further in cynomolgus monkey hepatocytes (Celsis Invitro Technology, Chicago, IL), which were grown in Dulbecco's modified Eagle's medium containing 10% FBS plus penicillin and streptomycin.

Cell Transfection. HepG2, DM1 myoblasts, and hSKMCs were transfected via electroporation in a 96-well plate format at 140 V (DM1 and hSKMCs) and 165 V (HepG2) with *DMPK* ASOs in a complete media (media plus 10% FBS) at room temperature. In general, *DMPK*-targeting ASOs with Gen 2.5 cEt chemistry were transfected at a 0.8 μ M concentration. The most effective ASOs from each chemical

class were further evaluated in dose-response experiments in hSKMCs, HepG2 cells, and patient DM1 muscle cells. After electroporation, cells were incubated overnight, and the following day, they were lysed in RLT buffer (Qiagen, Valencia, CA) for processing and further analysis. A similar transfection method was used for cynomolgus hepatocytes.

Fluorescence In Situ Hybridization. Ribonuclear inclusions were detected with a 5'-Cy3-labeled (CAG)₅ peptide nucleic acid probe (PNA Bio, Thousand Oaks, CA). DM1 cells were grown on gelatin-coated coverslips, fixed in 4% PFA fixation solution (in PBS, pH adjusted to 7.4) and incubated for 20 hours at 37°C in hybridization buffer (4 μ g/ μ l *Escherichia coli* transfer RNA; 5% dextran sulfate; 0.2% bovine serum albumin; 2 $\times$ SSC; 50% formamide; 2 mM vanadyl ribonucleoside complex; and 1 ng/ μ l peptide nucleic acid probe). The coverslips were then washed twice in 2 $\times$ SSC/50% formamide for 30 minutes at 37°C, stained with 5 μ M DRAQ5 (Thermo Scientific, Waltham, MA) for nucleus visualization, mounted on slides with Fluoromount (Sigma, St. Louis, MO), and sealed with generic clear nail polish. Cells were examined under a FluoView 300 confocal microscope (Olympus, Shinjuku, Japan) using argon-ion 488 nm, HeNe 543 nm, and HeNe 633 nm lasers. BA510IF + BA530RIF (green), 605BP (red), and BA660IF (far-red) filters and a PlanApo 60 $\times$ /1.4 oil $\approx$ 0.17 objective were used. Quantification of fluorescence in situ hybridization was performed using ImageJ 1.47 (US National Institute of Health, Bethesda, MD) (find maxima $\rightarrow$ segmented particles $\rightarrow$ noise tolerance 100–300).

Rodent Studies. The Institutional Animal Care and Use Committees at Isis and the University of Rochester approved all experiments. To evaluate efficacy against human *DMPK* (h*DMPK*) in mice, we used DMSXL transgenic mice, which feature a 45-kilobase human genomic fragment that includes *DMPK* with $\sim$ 800 or $>$ 1000 CTG repeats (Seznec et al., 2000; Huguet et al., 2012; Wheeler et al., 2012). Wild-type (WT) Balb/c (Charles River Laboratories, Wilmington, MA) and C57Bl6 (Jackson Laboratory, Sacramento, CA) mice served as controls.

ASO Selection and Animal Dosing. We designed several ASOs against the h*DMPK* transcript and evaluated them in hSKMCs and then in WT mice for changes in plasma chemistries for tolerability. ASOs that were tolerated in WT mice were evaluated for efficacy in DM1 transgenic mice ($n = 5$) by s.c. injection of 25 mg/kg twice weekly for 4–6 weeks. Forty-eight hours after the final dose, blood was drawn, and animals were sacrificed for tissue harvest. To determine the duration of the drug effect, we analyzed mice at 6, 15, and 31 weeks after the final dose. We also evaluated the tolerability of ISIS 486178 in Sprague-Dawley rats (Charles River Laboratories). Rats were administered ASO by s.c. injection at a dose of 50 mg/kg per week for 6 weeks. Blood was collected for analysis.

Gen 2.5 cEt *DMPK* ASO. The h*DMPK* ASO, ISIS 486178, is 16 residues in length and has a phosphorothioate backbone. The sequence is 5'-ACAATAAATACCGAGG-3'. Three nucleotides at the 5'- and 3'-ends have cEt modifications (underlined), and the central 10 nucleotides are deoxynucleotide sugars ("3–10–3 gapmer" design). It was designed to target the 3'-UTR region of the h*DMPK* transcript (Fig. 1A). The sequence of control ASO, a MOE gapmer, is 5'-CCTTCCCTGAAGGTTCTCTCC-3'.

ASO Safety and Efficacy in Cynomolgus Monkeys. We tested the pharmacologic activity and duration of action of ISIS 486178 in male cynomolgus monkeys. Saline ($n = 4$) or ISIS 486178 ($n = 8$; 40 mg/kg, 0.4 ml/kg dose volume) was administered by s.c. injection using a loading dose regimen on days 1, 3, 5, and 7 followed by a once-weekly maintenance dose for 12 additional weeks (total of 16 doses over 13 weeks). We selected this dose and treatment regimen based on previous experience with similar ASO therapeutics in monkeys.

During the treatment period, we monitored animal health by measuring body weight at regular intervals and serum chemistries; complete blood counts were determined after 1 month. To assess the ASO onset of action, muscle biopsies of the tibialis anterior were collected on day 44 (week 7) under slight sedation (0.1 ml/kg of

Fig. 1. Treatment with *DMPK*-targeting ISIS 486178 reduces *DMPK* mRNA in multiple human cell types and cynomolgus monkey hepatocytes. (A) Region of *hDMPK* targeted by ISIS 486178 relative to expanded CUG repeat in 3'-UTR. ISIS 486178 was electroporated into (B) HepG2 cells, (C) DM1/Steinert myoblasts (>1000 CTG repeats), and (D) cynomolgus monkey primary hepatocytes at the indicated concentrations. After 24 hours, cells were lysed and total RNA was isolated and human *DMPK* mRNA levels were determined by qPCR and normalized to total RNA. A control ASO was examined. Error bars represent standard errors of means ($n = 2$ to 3). *** $P < 0.001$ versus untreated control (UTC) using two-way analysis of variance. (E) Reduction of RNA foci in DM1 patient myoblasts upon treatment with *DMPK* ASO. DM1 myoblast cells were treated with ISIS 486178 at 20 nM concentration for 24–48 hours (bottom panels). Control treatment groups are shown in top panels. After the treatment, fluorescent in situ hybridization was performed on these cells. Nuclei were stained with DAPI (blue) and CUG^{exp} RNA foci (red).

ketamine) and local anesthetic (2% lidocaine) using 18-gauge needles (Bard Peripheral Vascular, Inc., Tempe, AZ). We also measured cardiac conduction events by electrocardiography recordings once

prior to treatment (week -1), and in weeks 12 [dosing group (total of 14 doses)] and 26 (recovery group) using a Cardio XP (Bionet Co., Ltd., Seoul, Korea). Twelve lead electrocardiogram (ECG) recordings

were made from restrained awake monkeys before ASO treatment and at day 79 ($n = 7$) and 87 days postdosing ($n = 3$). The wires with clips were connected to the animals in the monkey chair using the standard four limbs and six chest leads. The laboratory assistant restrained the monkey's arms and legs while the veterinarian performed the ECG recording.

On day 93 (week 13) of the treatment period, approximately 48 hours after the final dose, all four animals in the control group and half (four) of the ISIS 486178 treatment group were sacrificed, blood was collected, and a necropsy was performed. The remaining four animals in the ISIS 486178 group were monitored for another 13 weeks (26 weeks total), with tibialis anterior (TA) muscle biopsies on day 135 (week 19) and sacrifice and tissue harvest on day 182 (week 26). At necropsy, liver, kidney, heart, and skeletal muscle tissues from each animal were harvested and flash frozen in liquid nitrogen for determination of *DMPK* mRNA and tissue drug levels. Tissues were also fixed in 10% formalin and processed for histopathological evaluation.

RNA Isolation and Real-Time Polymerase Chain Reaction Analysis. Total RNA from cell culture experiments was prepared using the Qiagen RNeasy kit. Quantitative real-time polymerase chain reaction (PCR) was performed using the Qiagen QuantiTect Probe kit. Quantitative PCR (qPCR) reactions (20- μ l volumes) were run in duplicate and normalized to total RNA levels determined using the Ribogreen dye (Life Technologies).

For *in vivo* experiments, RNA was isolated by homogenizing tissues in RLT buffer (Qiagen), with 1% β -mercaptoethanol using either zirconium oxide beads (Next Advance, Inc., Averill Park, NY) or a hand-held homogenizer. The lysates were centrifuged overnight on a cesium chloride gradient at 35,000 rpm ($\sim$ 116,000g). The following day, RNA was purified using spin columns (Qiagen). Liver and kidney samples were processed using a hand-held homogenizer, and RNA was purified using spin columns (Qiagen) according to the manufacturer's protocol. Quantitative real-time PCR was performed using a custom-made reverse transcription PCR enzymes and reagents kit (Invitrogen). Primer and probe sets were designed with Primer Express Software (PE Applied Bioscience, Foster City, CA). The primers and probes used in the current studies were 1) for human/monkey *DMPK* mRNA analysis, forward primer 5'-AGCCT-GAGCCGGGAGATG-3', reverse primer 5'-GCGTAGTTGACTGGC-GAAGTT-3', and probe 5'-AGGCCATCCGCACGACAACC-3'; and 2) for mouse *DMPK* mRNA analysis, forward primer 5'-GACATATGC-CAAGATTGTGCACTAC-3', reverse primer 5'-CACGAATGAGGTCCT-GAGCTT-3', and probe 5'-AACACTTGTGCTGCCGCTGGC-3'.

Reactions were performed on an ABI Prism 7700 sequence detector (PE Applied Biosciences) or StepOne Real-Time PCR system (Applied Biosystems, Foster City, CA). For the analysis, 25 ng of total RNA was used for each reaction, and each sample was run in triplicate. Levels were normalized to total RNA levels (determined using a Ribogreen assay).

Immunostaining. Staining of the ASO in mouse tissues was performed as described previously (Hung et al., 2013). Briefly, a section of tissue was fixed in 10% neutral buffered formalin. Slides with tissues were incubated with a proprietary polyclonal rabbit anti-ASO primary antibody (Isis Pharmaceuticals, Carlsbad, CA) followed by incubation with goat anti-rabbit HRP secondary antibody (Jackson ImmunoResearch, West Grove, PA). To visualize the ASO, slides were developed with 3,3'-diaminobenzidine, followed by counter staining with hematoxylin (Surgipath, Leica Biosystems, Buffalo Grove, IL).

Blood Chemistry. Blood was collected into serum separator tubes (BD, Franklin Lakes, NJ) and centrifuged at low speed for 5 minutes. The serum supernatant was stored at -80°C . Plasma serum aspartate transaminase, alanine transaminase, blood urea nitrogen, creatinine, and creatine kinase values were determined using Olympus reagents and an Olympus AU400e analyzer.

Measurement of Drug Levels in Tissue. Each piece of tissue (about 50–100 mg) was weighed, and the amount of ASO was measured using several bioanalytical methods (Yu et al., 2013),

including capillary gel electrophoresis coupled with UV detection, high-performance liquid chromatography, and a hybridization-based enzyme-linked immunosorbent assay. The hybridization-based enzyme-linked immunosorbent assay probe was complementary to ISIS 486178 and contained a digoxigenin at the 5'-end and a biotin-triethylene glycol at the 3'-end.

Statistical Analysis. Data were analyzed by using an unpaired/paired *t* test with Welch's correction, one- or two-tailed Student's paired *t* test, or one- or two-way analysis of variance, followed by either Dunnett's or Bonferroni post-tests to detect the differences between or among groups. We used either GraphPad Prism (San Diego, CA) or Microsoft Excel (Redmond, WA) software to perform these analyses. A *P* value < 0.05 was considered statistically significant.

Results

Evaluation of ISIS 486178 Potency in Mouse, Monkey, and Human Cells. We designed more than 600 cEt-modified gapmer *DMPK* ASOs, with different lengths ranging from 14- to 17-mers to target both exonic and intronic regions of *hDMPK*. Our *in vitro* screening strategy first consisted of a single dose evaluation of the *DMPK* ASOs, followed by a dose-response evaluation of the most potent *DMPK* ASOs. A second cell line was also used to confirm the activity of the selected *DMPK* ASOs. After *in vitro* screening, we further evaluated the tolerability of the most potent *DMPK*-targeting ASOs in mice as well as in rats, leading to the identification of ISIS 486178. An attractive feature of ISIS 486178 is that this ASO is homologous to mouse, monkey, and human *DMPK* transcripts and thus can be employed to evaluate the effect of *DMPK* reduction across species.

To determine the potency of ISIS 486178 in reducing the levels of *DMPK* mRNA in cells from targeted species, we performed dose-response experiments in mouse, monkey, and human cells in culture. ISIS 486178 induced a dose-dependent reduction of *hDMPK* RNA levels in human HepG2 cells and DM1 patient myoblasts ($\text{IC}_{50} = 0.7$ and $0.5 \mu\text{M}$, respectively) following delivery to the cells by electroporation, whereas treatments with control ASO had no effect (Fig. 1, B and C). A dose of $0.8 \mu\text{M}$ ISIS 486178 also inhibited *hDMPK* mRNA levels by $\sim 90\%$ in non-DM1 hSKMCs (Supplemental Fig. 1). In primary monkey hepatocytes treated with ISIS 486178, a dose-dependent reduction of monkey *DMPK* mRNA expression was also observed ($\text{IC}_{50} < 0.06 \mu\text{M}$), and again the control ASO had no effect (Fig. 1D).

A molecular hallmark of DM1 pathology is the formation of nuclear foci containing the mutant *DMPK* CUG^{exp} RNA bound to RNA-binding proteins, such as MBLN1 (Davis et al., 1997; Miller et al., 2000). To determine whether ISIS 486178 treatment resulted in a decrease of nuclear foci numbers in cells from DM1 patients, we performed fluorescence *in situ* hybridization using a Cy3-labeled CAG probe and found a near complete elimination ($\sim 90\%$) of these foci from the nuclei of treated cells (Fig. 1E). Of note, *DMPK* mRNA levels were measured at 24 hours post-treatment, whereas nuclear foci were measured at 48 hours post-treatment.

In Vivo Evaluation of Systemically Administered ISIS 486178 in Wild-Type Mice and Rats. To explore the *in vivo* potential of ISIS 486178 to target *DMPK* in muscle, we first characterized its activity when administered systemically to mice. Importantly, ISIS 486178 was not formulated in any complex vehicle for these *in vivo* studies; it was prepared in saline solutions and administered systemically. C57Bl/6

Fig. 2. Localization and *DMPK* mRNA expression in tissues of wild-type mice after systemic administration of ISIS 486178. (A) ISIS 486178 was administered s.c. to normal C57Bl/6 mice ($n = 4$ per dose group) at 12.5 or 25 mg/kg body weight twice a week for 6 weeks. ASO levels in the liver, kidney, and quadriceps muscle were determined by immunostaining with anti-ASO antibody, followed by counterstaining with hematoxylin. (B) C57Bl/6 mice ($n = 4$ per dose group) were treated s.c. with PBS or ISIS 486178 twice weekly for 6 weeks at doses of 12.5 or 25 mg/kg body weight. Animals were sacrificed 48 hours after the final dose, and the liver and quadriceps muscle were collected. *DMPK* mRNA levels were measured by quantitative real-time PCR and normalized to total RNA. Error bars represent standard errors of means ($n = 3$ to 4). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ versus PBS-treated group using two-way analysis of variance, followed by Bonferonni multiple comparison tests.

mice were treated with 12.5 or 25 mg/kg body weight ISIS 486178 twice a week for 6 weeks by s.c. injection. Two days after the final dose, mice were sacrificed and tissues and blood were collected for further analysis. Distribution and accumulation of ASO in tissues was confirmed by immunohistochemistry with an anti-ASO antibody (Fig. 2A). ISIS 486178 treatment produced a robust and dose-dependent reduction in mouse *DMPK* (*mDMPK*) mRNA levels in both liver and skeletal muscle (quadriceps) (Fig. 2B). In a second mouse strain, BalbC mice, treatment with ISIS 486178 also resulted in >90% reduction of *mDMPK* mRNA levels in the quadriceps muscle and liver, with a similar safety profile as in C57Bl/6 mice (Supplemental Fig. 2; Supplemental Table 1). These data demonstrate that systemic delivery of a cEt-modified

DMPK-targeting ASO results in a more than 90% reduction of *mDMPK* mRNA expression in the skeletal muscle and liver of normal mice.

To determine the safety of pharmacological inhibition of *DMPK* in the adult animal, we performed several analyses on mice treated with ISIS 486178 for 6 weeks. Depletion of *DMPK* by ISIS 486178 was very well tolerated; body weights, organ weights, and serum chemistries were similar in ISIS 486178-treated and vehicle-treated mice (Table 1). Histopathological evaluations of the liver, kidney, spleen, and quadriceps muscle tissues of ASO-treated mice were all normal (Supplemental Fig. 3). Thus, the reduction of *mDMPK* mRNA levels by >90% by cEt *DMPK* ASO is safe and well tolerated in adult mice.

TABLE 1

Blood chemistries and tissue weights in wild-type normal C57Bl/6 mice following ISIS 486178 administration

Four mice per group were treated with 12.5 or 25 mg/kg body weight twice a week for 6 weeks. Values are expressed as means $\pm$ standard errors of means. There was no significant difference between PBS and ASO treatments on the blood chemistries and tissues weights using one-way analysis of variance, followed by Dunnett's multiple comparison tests.

Endpoint	Treatment Groups		
	PBS	12.5 mg/kg ISIS 486178	25 mg/kg ISIS 486178
ALT (IU/l)	33.25 $\pm$ 3.68	28.5 $\pm$ 0.29	32.5 $\pm$ 1.55
AST (IU/l)	60.75 $\pm$ 5.41	53.25 $\pm$ 8.29	46 $\pm$ 2.04
BUN (IU/l)	33.15 $\pm$ 3.53	25.925 $\pm$ 1.78	28.225 $\pm$ 1.52
CK (U/l)	134.5 $\pm$ 34.25	130.25 $\pm$ 41	89.25 $\pm$ 19.53
Creatinine (mg/dl)	0.1775 $\pm$ 0.02	0.1375 $\pm$ 0.01	0.145 $\pm$ 0.01
Hematocrit (HCT) (%)	49.25 $\pm$ 0.85	46.75 $\pm$ 1.11	46.25 $\pm$ 1.60
Neutrophil ($\times 10^3$)	2956.25 $\pm$ 972.86	2063.75 $\pm$ 1000.82	2068 $\pm$ 1030.04
Lymphocyte ($\times 10^3$)	6687.75 $\pm$ 377.86	7690.5 $\pm$ 424.42	5514 $\pm$ 711.37
Platelet ($\times 10^3$)	1078.75 $\pm$ 171.71	1335.75 $\pm$ 135.45	1490.75 $\pm$ 51.71
Tissue weights			
Liver (g)	1.36 $\pm$ 0.025	1.16 $\pm$ 0.178	1.64 $\pm$ 0.079
Spleen (g)	0.09 $\pm$ 0.007	0.11 $\pm$ 0.01	0.10 $\pm$ 0.004
Kidney (g)	0.33 $\pm$ 0.012	0.35 $\pm$ 0.017	0.34 $\pm$ 0.004

ALT, alanine transaminase; AST, aspartate transaminase; BUN, blood urea nitrogen; CK, creatine kinase.

Fig. 3. ISIS 486178 treatment reduced both human and endogenous mouse *DMPK* mRNA expression in DMSXL mice expressing the human *DMPK* gene with >1000 CTG repeats. DMSXL mice ($n = 5$ per group) were injected s.c. with PBS or ISIS 486178 at doses of 12.5, 25, and 50 mg/kg body weight twice a week for 6 weeks. Mice were sacrificed 2 days after the last dose. Organs and blood samples were collected postsacrifice. (A) ISIS 486178 was visualized in the liver, kidney, and quadriceps muscle by immunostaining with anti-ASO antibody, followed by counterstaining with hematoxylin. (B) Human and (C) mouse *DMPK* mRNA levels were quantified by quantitative real-time PCR and normalized to total RNA input. Error bars represent standard errors of means. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ versus PBS-treated group using one-way analysis of variance, followed by Bonferroni post-tests.

The additional safety of ISIS 486178 was evaluated in normal Sprague-Dawley rats. Rats were administered with either saline or ISIS 486178 at 50 mg/kg body weight per week for 6 weeks by s.c. injection. Analysis of plasma transaminases and blood urea nitrogen levels revealed no significant differences between saline and ISIS 486178 treatment (Supplemental Table 2). Tissue weights, such as the liver and kidney, were also not affected by ASO treatment; however, we found a significant increase in the spleen weight by ASO treatments, which was not unexpected as this is known to be a rat-specific class effect of oligonucleotides (Agrawal et al., 1997).

In Vivo Evaluation of *DMPK* ASO in DMSXL Mice. In DMSXL mice, the entire *hDMPK* gene is expressed as a transgene that contains between 800 and >1000 CTG repeats in the 3'-UTR region. Hemizygous DMSXL mice express low levels of the *hDMPK* transgene in the heart and skeletal muscle, exhibit normal pre-mRNA splicing, and do not exhibit myotonia (Huguet et al., 2012). The level of expression of the *hDMPK* transgene is lower than transcripts derived from the endogenous *mDMPK* gene in hemizygous mice (Supplemental Table 3). Using these mice, we evaluated the activity of ISIS 486178 against the human gene in vivo. Cohorts of male and female mice (ranging in age from 10 to 20 weeks) were treated with ISIS 486178 at 12.5, 25, or 50 mg/kg body weight twice weekly for 6 weeks. Analysis of ASO localization demonstrated that ISIS 486178 accumulation in tissues of DMSXL mice was similar to that in WT mice (Fig. 3A). qPCR analysis demonstrated a dose-dependent reduction of *hDMPK* mRNA expression in the heart (up to ~60%) and multiple skeletal muscles (up to 70% in the diaphragm) (Fig. 3B). Activity in the diaphragm is especially relevant as respiratory failure is a strong contributor to the mortality associated with DM1 (de Die-Smulders et al., 1998; Groh et al., 2008; Panaite et al., 2013). Since ISIS 486178 is crossreactive with mouse and human *DMPK* sequences, we evaluated levels of the endogenous *mDMPK* mRNA in DMSXL mice. ISIS 486178 treatment reduced levels of the *mDMPK* mRNA to a greater extent than it did *hDMPK* mRNA in all the muscles examined (Fig. 3C). In addition to this, the differences in sensitivity of the human and mouse transcripts may be attributed to differences in tertiary RNA structures of the mouse and human *DMPK* RNA,

resulting in differences in the local accessibility of the ASO to its target sites. Moreover, approximately 40% of the endogenous wild-type *DMPK* transcript is localized in the nucleus, which may also contribute to increased sensitivity to ASO treatment, as suggested above (Davis et al., 1997). ASO treatments were well tolerated in DMSXL mice, with serum chemistries all in the normal range (Table 2, top panel). Evaluation of drug levels in the liver and quadriceps muscle of these mice demonstrated that the liver had 18- to 20-fold more drug than muscle (Supplemental Table 4). Despite low drug levels in the muscle, the cEt-modified ASO significantly reduced targeted mRNA levels in muscle tissue.

ISIS 486178 Treatment Results in Prolonged Inhibition of *DMPK*. Previous studies have suggested that ASOs may have prolonged activity in postmitotic tissues, such as neurons and skeletal muscle, compared with tissues, such as liver, that have higher proliferation or regeneration rates (Hua et al., 2010; Wheeler et al., 2012; Lieberman et al., 2014; Rigo et al., 2014). To determine the duration of *DMPK* mRNA inhibition in tissues, we treated DMSXL mice with ISIS 486178 twice weekly for 6 weeks and determined endogenous *mDMPK* and *hDMPK* mRNA levels 2 days and 6, 15, and 31 weeks after the final dose. Reduction of *hDMPK* and *mDMPK* RNA levels were similar to those observed in the dose-response experiments at week 6 (Fig. 4). Levels of *hDMPK* mRNA were strongly inhibited in TA and the diaphragm over the 15-week treatment-free period. Prolonged pharmacologic activity of *DMPK* ASO was observed in several of the skeletal muscles evaluated, including the quadriceps, gastrocnemius, and diaphragm, where *hDMPK* mRNA expression remained below baseline levels at 31 weeks post-treatment (Fig. 4A). However, not all muscles showed this long-lived effect, for example, *DMPK* levels had returned to baseline in the tibialis anterior muscle at 31 weeks (Fig. 4). In the cardiac muscle, *hDMPK* inhibition began to reverse by 6 weeks and was back to baseline by 31 weeks. Furthermore, the wild-type *mDMPK* RNA levels were more strongly inhibited by ISIS 486178 treatment than the CUG^{exp} *hDMPK* RNA levels. However, the *mDMPK* levels appeared to recover more quickly than the *hDMPK* RNA levels (Fig. 4), suggesting that the duration of effect for the *hDMPK* RNA with the expanded CUG repeat is

TABLE 2

Blood parameters of DMSXL mice following ISIS 486178 treatment

Five mice per group were treated with ISIS 486178 at doses of 12.5, 25, and 50 mg/kg body weight twice a week for 6 weeks. Values are from five mice and are expressed as means $\pm$ standard errors of means. There was no significant difference between PBS and ASO treatments on the blood chemistries using one-way analysis of variance, followed by Dunnett's multiple comparison tests.

	ALT	AST	CK	Creatinine	BUN
	IU/l	IU/l	U/l	mg/dl	IU/l
PBS	32 $\pm$ 2.9	75.2 $\pm$ 21	224.8 $\pm$ 115.7	0.15 $\pm$ 0.01	28.74 $\pm$ 0.88
0 weeks (2 days after the last dose)	37.2 $\pm$ 6.04	72.8 $\pm$ 15.37	163.6 $\pm$ 53.19	0.118 $\pm$ 0.01	30.22 $\pm$ 1.68
6 weeks	26 $\pm$ 1.22	42.6 $\pm$ 4.04	76.6 $\pm$ 19.82	0.114 $\pm$ 0.01	26.26 $\pm$ 1.25
15 weeks	30.8 $\pm$ 1.07	56.4 $\pm$ 11.83	123.6 $\pm$ 53.01	0.136 $\pm$ 0.01	22.72 $\pm$ 2.33
31 weeks	39 $\pm$ 9.72	81 $\pm$ 19.60	206.8 $\pm$ 92.37	0.13 $\pm$ 0.01	25.38 $\pm$ 1.34
25 mg/kg per wk ISIS 486178	42 $\pm$ 11.9	67.4 $\pm$ 14.1	166.8 $\pm$ 78.8	0.164 $\pm$ 0.01	26.66 $\pm$ 2.72
50 mg/kg per wk ISIS 486178	35.4 $\pm$ 5	76.6 $\pm$ 25.4	218 $\pm$ 112	0.118 $\pm$ 0.01	24.82 $\pm$ 2.53
0 weeks (2 days after the last dose)	38.6 $\pm$ 7.33	62 $\pm$ 3.78	113.2 $\pm$ 21.14	0.108 $\pm$ 0.02	27.26 $\pm$ 3.283
6 weeks	24.2 $\pm$ 2.18	68.2 $\pm$ 19.78	166.6 $\pm$ 71.74	0.11 $\pm$ 0.01	26.66 $\pm$ 1.04
15 weeks	45.4 $\pm$ 9.97	97.4 $\pm$ 30.96	316.4 $\pm$ 129.46	0.2 $\pm$ 0.02*	31.1 $\pm$ 3.47
31 weeks	24.25 $\pm$ 1.31	72.75 $\pm$ 22.57	234.5 $\pm$ 94.84	0.110 $\pm$ 0.00	24.88 $\pm$ 1.40
100 mg/kg per wk ISIS 486178	30 $\pm$ 5.1	73 $\pm$ 25.3	193.2 $\pm$ 140.3	0.086 $\pm$ 0.02	38.48 $\pm$ 12.05

ALT, alanine transaminase; AST, aspartate transaminase; BUN, blood urea nitrogen; CK, creatine kinase.

* $P < 0.01$ PBS versus ASO treatments using one-way analysis of variance, followed by Bonferroni post-tests.

Fig. 4. Duration of action of ISIS 486178 in DMSXL mice. Four cohorts of 10- to 12-week-old DMSXL mice were injected subcutaneously with PBS or ISIS 486178 at a dose of 25 mg/kg of body weight twice weekly for 6 weeks ($n = 5$). Groups were sacrificed 2 days and 6, 15, and 31 weeks after the last dose. Heart, TA, quadriceps (Quad), gastrocnemius (Gastroc), and diaphragm tissues were harvested. RNA was isolated, and quantitative real-time PCR was performed to determine levels of (A) hDMPK and (B) mDMPK mRNAs. Error bars represent standard errors of means. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ versus PBS-treated groups using two-way analysis of variance, followed by Bonferroni multiple comparison test.

somewhat longer than for wild-type mDMPK. To further compare the rebound kinetics of the mouse or human DMPK transcripts, we performed additional analyses of the post-treatment time RNA recovery kinetics at week 0 (day 2), 6, and 15 for each muscle type (Supplemental Fig. 4, A and B; Supplemental Table 5). The rebound rates of expression of mDMPK and hDMPK were in fact different, with the rate of recovery for the human CUG^{exp} DMPK transcript being slower than that of the mDMPK transcript ($P = 0.04$; Supplemental Table 5). These results suggest that ASO-mediated inhibition of mutant DMPK in DM1 may have prolonged activity. Additionally, ISIS 486178 treatment was generally well tolerated; however, plasma creatinine levels increased at week 15 but returned within a normal range by week 31 (Table 2, bottom panel).

Systemic Administration of ISIS 486178 Reduces Skeletal Muscle DMPK RNA Levels in Cynomolgus Monkeys. The complementarity of ISIS 486178 across species allowed the characterization of its pharmacologic activity

and safety profile in nonhuman primates as well as rodents. Cynomolgus monkeys were treated with 40 mg/kg ISIS 486178 at day 1, 3, 5, and 7 and then weekly to complete 13 weeks of treatment. ASO tolerability, pharmacologic activity, onset, and duration of actions were evaluated. Treatment with ISIS 486178 was well tolerated, with no clinical observations, and the body weights and serum chemistries of ASO-treated animals were in the normal range (Table 3). Histologic evaluation of tissues showed no evidence of drug-induced effects in the tissues (Supplemental Fig. 5). Tissue weights were also within normal range; however, we observed a minor increase in the liver weights of the treated animals, which was found to be significant (Supplemental Fig. 6) but was within the normal range. Measurement of DMPK transcripts by qPCR showed a 50–70% reduction of DMPK mRNA in several muscle tissues (Fig. 5A). Evaluation of tissues for full-length (undergraded) ASO demonstrated that the drug was detected in all tissues examined even at 13 weeks after the end of the dosing period (Supplemental Table 6). To assess the onset of

TABLE 3

Pre- and post-DMPK ASO ISIS 486178 treatment mean serum chemistry parameters in cynomolgus monkeys

Male cynomolgus monkeys were treated s.c. with PBS or ISIS 486178 on days 1, 3, 5, and 7 and once weekly for another 12 weeks (13 weeks total). Values are from four monkeys and expressed as means $\pm$ standard errors of means. There was no significant difference between saline and ASO treatments for the predose and postdose on the blood chemistries' value using one-way analysis of variance, followed by Bonferroni multiple comparison tests.

Endpoint	Treatment	Predose		Postdose	
		Day -14	Day -7	Day 30	Day 93
BUN (mg/dl)	Saline	22.65 $\pm$ 1.90	24.20 $\pm$ 2.24	22.45 $\pm$ 1.50	23.06 $\pm$ 1.42
	ASO	23.97 $\pm$ 1.04	27.07 $\pm$ 1.61	22.05 $\pm$ 1.28	23.93 $\pm$ 0.94
AST (U/l)	Saline	34.20 $\pm$ 3.86	38.75 $\pm$ 3.15	43.00 $\pm$ 5.08	66.08 $\pm$ 10.99
	ASO	37.64 $\pm$ 2.74	49.78 $\pm$ 5.80	47.04 $\pm$ 6.86	59.70 $\pm$ 7.77
ALT (U/l)	Saline	25.90 $\pm$ 4.50	27.75 $\pm$ 5.22	34.38 $\pm$ 5.42	42.98 $\pm$ 8.10
	ASO	40.48 $\pm$ 6.16	54.98 $\pm$ 10.19	41.15 $\pm$ 4.35	43.59 $\pm$ 5.84
CK (U/l)	Saline	160.75 $\pm$ 11.64	249.00 $\pm$ 30.48	300.00 $\pm$ 114.49	898.75 $\pm$ 330.11
	ASO	236.75 $\pm$ 48.14	380.75 $\pm$ 81.59	415.00 $\pm$ 162.69	809.57 $\pm$ 168.76
CRP (mg/l)	Saline	0.22 $\pm$ 0.04	0.23 $\pm$ 0.03	0.24 $\pm$ 0.03	0.35 $\pm$ 0.08
	ASO	0.19 $\pm$ 0.04	0.18 $\pm$ 0.03	0.20 $\pm$ 0.03	0.21 $\pm$ 0.03
RBC ($\times 10^6$)	Saline	6.13 $\pm$ 0.16	5.74 $\pm$ 0.14	5.86 $\pm$ 0.05	5.59 $\pm$ 0.17
	ASO	6.00 $\pm$ 0.16	5.70 $\pm$ 0.11	6.03 $\pm$ 0.15	6.07 $\pm$ 0.17
WBC ($\times 10^3$)	Saline	8.83 $\pm$ 0.56	10.50 $\pm$ 0.96	10.65 $\pm$ 0.42	11.44 $\pm$ 0.48
	ASO	13.40 $\pm$ 1.94	13.46 $\pm$ 1.66	13.05 $\pm$ 1.37	12.62 $\pm$ 1.65
Platelets ($\times 10^3$)	Saline	398.25 $\pm$ 29.89	461.75 $\pm$ 20.53	395.00 $\pm$ 18.66	396.50 $\pm$ 32.97
	ASO	428.75 $\pm$ 34.67	472.13 $\pm$ 32.73	438.00 $\pm$ 43.61	422.71 $\pm$ 44.79
RETA ($\times 10^3/\text{mm}^3$)	Saline	65.88 $\pm$ 13.10	74.38 $\pm$ 10.44	71.93 $\pm$ 14.98	56.53 $\pm$ 5.36
	ASO	70.64 $\pm$ 5.88	78.88 $\pm$ 4.96	82.51 $\pm$ 6.51	51.89 $\pm$ 3.50

ALT, alanine transaminase; AST, aspartate transaminase; BUN, blood urea nitrogen; CK, creatine kinase; CRP, C-reactive protein; RBC, red blood cell; WBC, white blood cell.

action of ISIS 486178 treatment and duration of action post-treatment, we biopsied TA muscles in live animals (under mild anesthesia) after 7 weeks of the 13-week treatment period and then at 19 and 26 weeks (7 and 13 weeks post-treatment). Evaluation of this biopsy sample revealed an 80% reduction of *DMPK* mRNA as early as week 7 of the treatment (Fig. 5B). A subgroup of animals was necropsied 13 weeks post-treatment (week 26) to characterize the duration of action. At necropsy, we collected several additional muscle groups (Fig. 5A). Thirteen weeks after the last dose, *DMPK* mRNA levels were 60% of those in control in TA, quadriceps, and deep flexor muscles, whereas in other muscles, *DMPK* levels had returned to pretreatment levels, e.g., biceps, deltoid, tongue, heart, liver, and kidney (Fig. 5A).

In light of the cardiac conduction block reported in aged *DMPK*-deficient mice (Berul et al., 1999, 2000), we performed electrocardiographic measurements before (week -1) and after 12 weeks of ISIS 486178 dosing (14 doses) as well as in animals in the recovery group [in week 26 (13-week treatment-free period)]. The ECG recordings showed no significant changes in the cardiac conduction intervals with *DMPK* ASO treatment, suggesting that a 50% reduction of *DMPK* mRNA in heart tissue over 13 weeks does not have a major functional impact on cardiac conduction in adult nonhuman primates (Supplemental Table 7).

Discussion

To date, no therapies have been identified that target the underlying molecular pathology of DM1, which is caused by expression of *DMPK* CUG^{exp}, a toxic CUG repeat-containing RNA. ASOs are an emerging class of therapeutics that enables specific inhibition of previously undruggable disease targets. In this study, we have characterized the in vitro and in vivo activity of a prototype cEt-modified ASO targeted to the *DMPK* mRNA. ISIS 486178 was well tolerated and inhibited

expression of *DMPK* RNA in skeletal and cardiac muscle of the mouse and monkey, tissues clinically involved in DM1. Despite sustained inhibition of endogenous *DMPK* expression for several weeks, however, muscle histology appears normal in multiple strains of mice and nonhuman primates, which is consistent with the mild phenotype observed in *DMPK*-knockout mice (Jansen et al., 1996; Reddy et al., 1996). Furthermore, the mild cardiac conduction abnormalities, which were reported in older adult *DMPK*^{+/-} and *DMPK*^{-/-} mice (Berul et al., 1999, 2000) were not observed in adult monkeys. The normal serum chemistries post-treatment further support the safety of *DMPK* reduction.

These results demonstrate that cEt-modified ASOs can be effectively employed to target genes expressed in extrahepatic tissues, such as skeletal muscle. Another example where skeletal muscle plays an important role in the pathogenesis is spinal and bulbar muscular atrophy, a disease in which a polyglutamine tract in the amino terminus of the androgen receptor (AR) is the underlying contributing entity. It has been recently reported that a cEt-containing ASO targeting AR is effective in reducing the AR mRNA levels in the skeletal muscle of a mouse model of spinal and bulbar muscular atrophy, resulting in phenotypic rescue (Lieberman et al., 2014). Importantly, both of these ASOs were administered subcutaneously in saline solution, whereas other nucleic acid-based therapies, such as those employing small interfering RNAs, require formulation in delivery vehicles or conjugation to ligands that mediate cell uptake (Wei et al., 2011; Wen and Meng, 2014; Zhou et al., 2014).

ISIS 486178 inhibited *DMPK* transcript expression by about 80% in several cell lines and, importantly, substantially eliminated RNA foci in patient-derived DM1 myoblast cells. Furthermore, reduction of *DMPK* mRNA levels in mice and monkeys after subcutaneous dosing was sustained for several weeks after the cessation of dosing, consistent with our previous findings in mouse models of DM1 and spinal

Fig. 5. ISIS 486178 reduces *DMPK* mRNA expression in normal cynomolgus monkey tissues, with a prolonged duration of action in TA muscle. (A) Male monkeys ($n = 4$ per group) received s.c. injections of PBS or 40 mg/kg ISIS 486178 on days 1, 3, 5, and 7 and once weekly for another 12 weeks (13 weeks total). Several tissues were isolated 2 days after the last dose. Another group of monkeys dosed similarly was followed for 26 weeks. *DMPK* mRNA was measured by quantitative real-time PCR and normalized to total RNA levels. Quad, quadriceps; Gastroc, gastrocnemius. (B) Male monkeys ($n = 4$ per group) received s.c. injections of PBS or ISIS 486178 at days 1, 3, 5, and 7 and once weekly for another 12 weeks. TA muscle was biopsied at week 7 (during treatment) and week 19 (6 weeks post-treatment) as well as harvested at necropsy at week 13 (completion of the treatment) and 26 (13 weeks post-treatment). *DMPK* mRNA was measured by quantitative real-time PCR and normalized to total RNA levels. Error bars represent standard errors of means. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ versus PBS-treated groups using one-way analysis of variance, followed by Dunnett's multiple comparison test.

muscular atrophy. (Hua et al., 2010; Wheeler et al., 2012; Rigo et al., 2014). The prolonged ASO duration of action in the muscle and neurons may relate to the persistence of therapeutic drug levels within postmitotic tissues due to infrequent cell proliferation, although the precise mechanism remains to be determined. Consistent with this, we previously demonstrated prolonged activity in muscle as compared with the liver, with an ASO that targets *Malat1*, a nuclear-retained noncoding RNA transcript in muscle (Wheeler et al., 2012).

In the current study, the cross-species *DMPK* ASO induced greater knockdown of the *mDMPK* transcript than the *hDMPK* transcript in muscles of DMSXL mice. Previously, we reported that nuclear-retained transcripts appear to be more sensitive to RNase H ASOs (Wheeler et al., 2012). However, the current study does not permit a direct comparison of ASO sensitivity for nuclear-exported versus nuclear-retained transcripts because the *hDMPK* transcript is expressed from a transgene integration at much lower levels

than endogenous mDMPK. Additionally, the endogenous DMPK transcript may have a longer nuclear residence time than most protein-coding RNAs (Davis et al., 1997). Further investigation is needed to delineate the precise mechanisms underlying the prolonged duration of action of the ASO in muscle compared with other tissues and whether MOE or cEt chemistries differ in their efficiency of nuclear RNA targeting.

Other oligonucleotide-based therapeutic strategies have been designed to target mutant DMPK-CUG^{exp}, including direct targeting of the expanded CUG repeat sequences with CUG RNA-targeting drugs (Mulders et al., 2009; Wheeler et al., 2009; Lee et al., 2012). These approaches have been evaluated either in cultured cells or mice after intramuscular local administration. Local administration restricts the systemic distribution of ASOs and thus is less clinically applicable. CAG-containing morpholino ASOs conjugated to cell-penetrating peptides delivered intravenously have shown activity in transgenic mouse models of DM1 (Leger et al., 2013). However, the conjugation of ASO to a peptide moiety may facilitate muscle uptake but may also result in a greater risk of nephrotoxicity (Blake et al., 2002; Moulton and Moulton, 2010; Wu et al., 2012). Small molecule and peptide inhibitors have also been explored for the treatment of DM1 (Warf et al., 2009; Garcia-Lopez et al., 2011; Childs-Disney et al., 2012; Ofori et al., 2012; Hoskins et al., 2014). These inhibitors prevent the abnormal interaction between the CUG repeat-containing RNA and the splicing factor MBLN1. Although these results are encouraging, it is early in their development and additional work will be needed to further optimize the compounds for safety and potency.

In contrast to the above strategies, the ASO characterized here targets a region in the DMPK transcript that lies outside the CUG repeat region. Earlier studies with second-generation MOE ASOs suggested that muscle cell transcripts that are retained in the nucleus are more readily targeted than those that are exported to the cytoplasm (Wheeler et al., 2012). As the transcript that has toxicity is retained in the nucleus, DM1 is an ideal candidate for the ASO therapeutic strategy.

Taken together, these results indicate that cEt ASOs can be effectively and safely employed to therapeutically inhibit gene expression in muscle and that cEt ASOs targeting DMPK have potential as therapeutics to treat DM1 patients.

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Authorship Contributions

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