TARGETED GENE THERAPY FOR DM-1 MYOTONIC DYSTROPHY

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. § 119 (e) to U.S. Provisional Application Serial No. 63/328,241, filed April 6, 2022 and U.S. Provisional Application Serial No. 63/483,075, filed February 3, 2023, the contents of which are incorporated by reference herein.

SUBMISSION OF SEQUENCE LISTING

[0001] The content of the following submission in XML file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 737870_SA9-363PC_ST26.xml, date created: April 3, 2023, size: 82,900 bytes).

FIELD

[0002] The present invention relates to variant RNAi molecules. In some aspects, the invention relates to variant RNAi molecules to treat muscular dystrophy.

BACKGROUND

[0003] RNA interference (RNAi) has been shown to be a useful tool for gene silencing in basic research of gene function and shows great promise as a therapeutic agent to suppress genes associated with the development of a number of diseases. In nature, gene regulation by RNAi occurs through small RNAs known as microRNAs (miRNAs) (Ambros, (2004) *Nature* 431:350-355; Krol *et al.*, (2010) *Nat. Rev. Genet.* 11:597-610). MicroRNAs have emerged as powerful regulators of diverse cellular processes, and when delivered by viral vectors, artificial miRNAs are continually expressed, resulting in a robust and sustained suppression of target genes. The elucidation of the mechanisms involved in miRNA processing has allowed scientists to co-opt the endogenous cellular RNAi machinery and direct the degradation of a target gene product with the use of artificial miRNAs (see, *e.g.*, US PG Pub. 2014/0163214 and Davidson *et al.*, (2012) *Cell* 150:873-875).

[0004] Myotonic Dystrophy Type-1 (DM1) is a monogenic, autosomal-dominant, progressive disease caused by expansion of CTG repeats (>50) in the DMPK locus. The DMPK with repeats is transcribed into mRNA, which forms hairpins and binds RNA binding proteins, sequestering them from their normal function. This leads to the appearance of nuclear foci, mis-splicing of mRNAs, and ultimately myotonia. DM1 principally affects skeletal, cardiac and

smooth muscle, resulting in significant physical, cognitive and behavioral impairments and disability. There is currently no approved therapy for DM1. Therefore, there is a high unmet medical need for therapies to treat DM1.

[0005] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

BRIEF SUMMARY

[0006] In some aspects, the invention provides an RNAi comprising a first strand and a second strand, wherein a) the first strand and the second strand form a duplex; b) the first strand comprises a guide region, wherein the guide region comprises nucleic acid with the sequence 5'-AGUCGAAGACAGUUCUAGGGU-3' (SEQ ID NO:1) or with a sequence with about 90% identity to the sequence of SEQ ID NO:1; and c) the second strand comprises a non-guide region. In some embodiments, the non-guide region comprises nucleic acid with the sequence 5' ACCCUAGAUGUCUUCGAUU-3' (SEQ ID NO:2) or a with a sequence with about 90% identity to the sequence of SEQ ID NO:2. In some embodiments, the first strand comprises nucleic acid with the sequence of SEQ ID NO:1 and the non-guide region comprises nucleic acid with the sequence of SEQ ID NO:2. In some embodiments, the first strand and the second strand are linked by means of a RNA linker capable of forming a loop structure. In some embodiments, the RNA linker comprises from about 4 to about 50 nucleotides. In some embodiments, the loop structure comprises from about 4 to about 20 nucleotides. In some embodiments, the loop structure comprises nucleic sequence with of SEQ ID NO:3 or with a sequence with about 90% identity to the sequence of SEQ ID NO:3. In some embodiments, the RNAi comprises 5' to 3' the second strand, the RNA linker, and the first strand. In some embodiments, the RNAi comprises 5' to 3' the first strand, the RNA linker, and the second strand. In some embodiments, the RNAi comprises nucleic acid with the sequence of SEQ ID NO:7 or with a sequence with about 90% identity to the sequence of SEQ ID NO:7. In some embodiments, the RNAi is a small inhibitory RNA (siRNA), a microRNA (miRNA), or a small hairpin RNA (shRNA).

[0007] In some embodiments of the invention, the RNAi further comprises a scaffold. In some embodiments, the scaffold comprises all or a portion of the nucleic acid of SEQ ID No: 11. In some embodiments, the miRNA is embedded within the scaffold. In some embodiments, the scaffold has a 5'arm, wherein the 5' arm is located 5' to the nucleic acid encoding the RNAi, and a 3'arm, wherein the 3' arm is located 3' to the nucleic acid encoding the RNAi. In some embodiments, the scaffold is a miR-155 scaffold. In some embodiments, the miR-155 scaffold comprises the nucleic acid of SEQ ID NO:9 or a sequence with about 90% identity to the

sequence of SEQ ID NO:9 located 5' to the RNAi. In some embodiments, the miR-155 scaffold comprises the nucleic acid of SEQ ID NO:10 or a sequence with about 90% identity to the sequence of SEQ ID NO:10 located 3' to the RNAi.

[0008] In some embodiments of the invention, the RNAi targets RNA encoding a polypeptide associated with myotonic dystrophy-1 (DM1). In some embodiments, the polypeptide is dystrophia myotonica protein kinase (DMPK). In some embodiments, the DMPK comprises a mutation associated with DM1. In some embodiments, the gene encoding DMPK comprises five or more CTG trinucleotide repeats.

[0009] In some aspects, the invention provides an expression cassette comprising nucleic acid encoding any of the RNAi described herein. In some embodiments, the nucleic acid encoding the RNAi is operably linked to a promoter. In some embodiments, the promoter is a muscle-specific promotor. In some embodiments, the promoter is a desmin promoter or variant thereof. In some embodiments, the desmin promoter comprises one or more enhancer elements and the promoter for the human desmin gene. In some embodiments, the desmin promoter comprises two enhancer elements and the promoter for the human desmin gene. In some embodiments, the desmin promoter comprises one or more Byrne enhancer elements and/or one or more Paulin enhancer elements. In some embodiments, the desmin promoter comprises one or more enhancer elements comprising the nucleotide sequence of SEQ ID NO:21 or a nucleotide sequence with about 90% identity to the sequence of SEQ ID NO:21 and/or one or more enhancer elements comprising the nucleotide sequence of SEQ ID NO:22 or a nucleotide sequence with about 90% identity to the sequence of SEQ ID NO:22. In some embodiments, the desmin promoter comprises the nucleotide sequence of SEQ ID NO:12 or a sequence with about 90% identity to the nucleotide sequence of SEQ ID NO:12. In some embodiments, the expression cassette further comprises an intron. In some embodiments, the intron is a rabbit β-globin intron. In some embodiments, the intron comprises the nucleotide sequence of SEQ ID NO:13 or a sequence with about 90% identity to the sequence of SEQ ID NO:13. In some embodiments, the nucleic acid encoding the RNAi is embedded in the intron. In some embodiments, the intron comprises a 5' arm and a 3' arm, wherein the 5' arm is located 5' to the nucleic acid encoding the RNAi and the 3' arm is located 3' to the nucleic acid encoding the RNAi. In some embodiments, the 5' arm of the intron comprises the nucleotide sequence of SEQ ID NO:14 or a sequence with about 90% identity to the sequence of SEQ ID NO:14. In some embodiments, the 3' arm of the intron comprises the nucleotide sequence of SEQ ID NO:15 or a sequence with about 90% identity to the sequence of SEQ ID NO:15. In some embodiments, the expression cassette further comprises a polyadenylation signal. In some embodiments, the polyadenylation signal is a bovine

growth hormone polyadenylation signal, an SV40 polyadenylation signal, or a HSV TK pA. In some embodiments, the polyadenylation signal is a minimal bovine growth hormone polyadenylation signal. In some embodiments, the bovine growth hormone polyadenylation signal comprises the nucleotide sequence of SEQ ID NO:16 or a sequence with about 90% identity to the sequence of SEQ ID NO:16. In some embodiments, the expression cassette comprises the nucleotide sequence of SEQ ID NO:17 or a sequence with about 90% identity to the sequence of SEQ ID NO:17.

[0010] In some aspects, the invention provides an expression cassette, wherein the expression cassette comprises a modified desmin promoter, wherein the modified desmin promoter comprises one or more enhancer elements and the promoter for the human desmin gene. In some embodiments, the modified desmin promoter comprises two enhancer elements and the promoter for the human desmin gene. In some embodiments, the modified desmin promoter comprises one or more Byrne enhancer elements and/or one or more Paulin enhancer elements. In some embodiments, the modified desmin promoter comprises one or more enhancer elements comprising the nucleotide sequence of SEQ ID NO:21 or a nucleotide sequence with about 90% identity to the sequence of SEQ ID NO:21 and/or one or more enhancer elements comprising the nucleotide sequence of SEQ ID NO:22 or a nucleotide sequence with about 90% identity to the sequence of SEQ ID NO:22. In some embodiments, the desmin promoter comprises the nucleotide sequence of SEQ ID NO:12 or a sequence with about 90% identity to the nucleotide sequence of SEQ ID NO:12. In some embodiments, the expression cassette further comprises an intron. In some embodiments, the intron is a rabbit β-globin intron. In some embodiments, the intron comprises the nucleotide sequence of SEQ ID NO:13 or a sequence with about 90% identity to the sequence of SEQ ID NO:13. In some embodiments, the nucleic acid encoding the transgene is embedded in the intron. In some embodiments, the intron comprises a 5' arm and a 3' arm, wherein the 5' arm is located 5' to the nucleic acid encoding the transgene and the 3' arm is located 3' to the nucleic acid encoding the transgene. In some embodiments, the 5' arm of the intron comprises the nucleotide sequence of SEQ ID NO:14 or a sequence with about 90% identity to the sequence of SEQ ID NO:14. In some embodiments, the 3' arm of the intron comprises the nucleotide sequence of SEQ ID NO:15 or a sequence with about 90% identity to the sequence of SEQ ID NO:15. In some embodiments, the expression cassette further comprises a polyadenylation signal. In some embodiments, the polyadenylation signal is a bovine growth hormone polyadenylation signal, an SV40 polyadenylation signal, or a HSV TK pA. In some embodiments, the polyadenylation signal is a minimal bovine growth hormone polyadenylation signal. In some embodiments, the bovine growth hormone polyadenylation signal comprises the

nucleotide sequence of SEQ ID NO:16 or a sequence with about 90% identity to the sequence of SEQ ID NO:16. In some embodiments, the transgene encodes a polypeptide or a nucleic acid. In some embodiments, the transgene encodes an RNAi.

[0011] In some aspects, the invention provides a vector comprising any of the expression cassettes described herein. In some embodiments, the expression cassette is flanked by one or more stuffer nucleic acid sequences. In some embodiments, the one or more stuffer nucleic acid sequences is derived from the human SerpinA1 gene. In some embodiments, a stuffer nucleic acid sequence located 5' to the expression cassette is derived from the human SerpinA1 gene. In some embodiments, a stuffer sequence located 5' to the expression cassette comprises the nucleotide sequence of SEQ ID NO:18 or a sequence with about 90% identity to the sequence of SEQ ID NO:18. In some embodiments, a stuffer nucleic acid sequence located 3' to the expression cassette is derived from the human SerpinA1 gene. In some embodiments, a stuffer sequence located 3' to the expression cassette comprises the nucleotide sequence of SEQ ID NO:19 or a sequence with about 90% identity to the sequence of SEQ ID NO:19.

[0012] In some embodiments of the invention, the vector is a recombinant adeno-associated virus (rAAV) vector. In some embodiments, the expression cassette is flanked by one or more AAV inverted terminal repeat (ITR) sequences. In some embodiments, the expression cassette is flanked by two AAV ITRs. In some embodiments, the AAV ITRs are AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV2R471A, AAV DJ, a goat AAV, bovine AAV, or mouse AAV serotype ITRs. In some embodiments, the AAV ITRs are AAV2 ITRs. In some embodiments, the rAAV vector comprises the nucleotide sequence of SEQ ID NO:20 or a sequence with about 90% identity to the sequence of SEQ ID NO:20. In some embodiments, the vector is a self-complementary rAAV vector.

[0013] In some embodiments, the invention provides a cell comprising any of the expression cassette described herein, any of the vectors described herein, or any of the rAAV vectors described herein.

[0014] In some aspects, the invention provides a viral particle comprising any of the vectors described herein. In some aspects, the invention provides a recombinant AAV particle comprising any of the rAAV vectors described herein. In some embodiments, the AAV viral particle comprises an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAVrh74, AAVrh74 N502I, AAVrh74 W505R, AAV2R471A, AAV2/2-7m8, AAV DJ, AAV2 N587A, AAV2 E548A,

AAV2 N708A, AAV V708K, AAV2-HBKO, AAVDJ8, AAVPHP.B, AAVPHP.eB, AAVBR1, AAVHSC15, AAVHSC17, a goat AAV, AAV1/AAV2 chimeric, bovine AAV, or mouse AAV capsid rAAV2/HBoV1 serotype capsid. In some embodiments, the ITR and the capsid of the rAAV viral particle are derived from the same AAV serotype. In some embodiments, the ITR and the capsid of the rAAV viral particle are derived from different AAV serotypes. In some embodiments, the AAV viral particle comprises a AAVrh74 N502I serotype capsid. In some embodiments, the ITR is an AAV2 ITR and the capsid of the rAAV particle is an AAVrh74 N502I serotype capsid. In some embodiments, the AAV viral particle comprises a AAVrh74 W505R serotype capsid. In some embodiments, the ITR is an AAV2 ITR and the capsid of the rAAV particle is an AAVrh74 W505R serotype capsid. In some embodiments, the invention provides a rAAV particle comprising an rAAV vector and a capsid, wherein the rAAV vector comprises the following nucleic acids 5' to 3', an AAV2 ITR, nucleic acid encoding a stuffer nucleic acid sequence from the human serpinA1 gene, a Byrne desmin enhancer element, a Paulin desmin enhancer element, a desmin promoter, a 5' arm of a rabbit β-globin intron, a 5' miR155 scaffold sequence, a $DMPK^{204}$ miRNA guide sequence, a miR155 terminal loop sequence, a $DMPK^{204}$ miRNA passenger sequence, a 3' miR155 scaffold sequence, a 3' arm of a rabbit β-globin intron, a minimal bovine growth hormone polyadenylation sequence, nucleic acid encoding a stuffer nucleic acid sequence from the human serpinA1 gene, and an AAV2 ITR; and wherein the capsid is an AAVrh74 N502I capsid. In some embodiments, the invention provides a rAAV particle comprising an rAAV vector, wherein the rAAV vector comprises the following nucleic acids 5' to 3', an AAV2 ITR comprising the polynucleotide sequence of SEQ ID NO:43, nucleic acid encoding a stuffer nucleic acid sequence from the human serpinA1 gene comprising the polynucleotide sequence of SEQ ID NO:18, a Byrne desmin enhancer element comprising the polynucleotide sequence of SEQ ID NO:21, a Paulin desmin enhancer element comprising the polynucleotide sequence of SEQ ID NO:22, a desmin promoter comprising the polynucleotide sequence of SEQ ID NO:23, a 5' arm of a rabbit β-globin intron comprising the polynucleotide sequence of SEQ ID NO:14, a 5' miR155 scaffold sequence comprising the polynucleotide sequence of SEQ ID NO:40, a $DMPK^{204}$ miRNA guide sequence comprising the polynucleotide sequence of SEQ ID NO:4, a miR155 terminal loop sequence comprising the polynucleotide sequence of SEQ ID NO:6, a $DMPK^{204}$ miRNA passenger sequence comprising the polynucleotide sequence of SEQ ID NO:5, a 3' miR155 scaffold sequence comprising the polynucleotide sequence of SEQ ID NO:41, a 3' arm of a rabbit β-globin intron comprising the polynucleotide sequence of SEQ ID NO:15, a minimal bovine growth hormone polyadenylation sequence comprising the polynucleotide sequence of SEQ ID NO:16, nucleic acid encoding a

stuffer nucleic acid sequence from the human serpinA1 gene comprising the polynucleotide sequence of SEQ ID NO:19, and an AAV2 ITR comprising the polynucleotide sequence of SEQ ID NO:49; and wherein the capsid is an AAVrh74 N502I capsid. In some embodiments, the AAVrh74 N502I capsid comprises capsid proteins comprising the amino acid sequence of SEQ ID NO:50.

[0015] In some embodiments, the invention provides a rAAV particle comprising an rAAV vector and a capsid, wherein the rAAV vector comprises the following nucleic acids 5' to 3', an AAV2 ITR, nucleic acid encoding a stuffer nucleic acid sequence from the human serpinA1 gene, a Byrne desmin enhancer element, a Paulin desmin enhancer element, a desmin promoter, a 5' arm of a rabbit β-globin intron, a 5' miR155 scaffold sequence, a $DMPK^{204}$ miRNA guide sequence, a miR155 terminal loop sequence, a $DMPK^{204}$ miRNA passenger sequence, a 3' miR155 scaffold sequence, a 3' arm of a rabbit β-globin intron, a minimal bovine growth hormone polyadenylation sequence, nucleic acid encoding a stuffer nucleic acid sequence from the human serpinA1 gene, and an AAV2 ITR; and wherein the capsid is an AAVrh74 W505R capsid. In some embodiments, the invention provides a rAAV particle comprising an rAAV vector, wherein the rAAV vector comprises the following nucleic acids 5' to 3', an AAV2 ITR comprising the polynucleotide sequence of SEQ ID NO:43, nucleic acid encoding a stuffer nucleic acid sequence from the human serpinA1 gene comprising the polynucleotide sequence of SEQ ID NO:18, a Byrne desmin enhancer element comprising the polynucleotide sequence of SEQ ID NO:21, a Paulin desmin enhancer element comprising the polynucleotide sequence of SEQ ID NO:22, a desmin promoter comprising the polynucleotide sequence of SEQ ID NO:23, a 5' arm of a rabbit β-globin intron comprising the polynucleotide sequence of SEQ ID NO:14, a 5' miR155 scaffold sequence comprising the polynucleotide sequence of SEQ ID NO:40, a $DMPK^{204}$ miRNA guide sequence comprising the polynucleotide sequence of SEQ ID NO:4, a miR155 terminal loop sequence comprising the polynucleotide sequence of SEQ ID NO:6, a $DMPK^{204}$ miRNA passenger sequence comprising the polynucleotide sequence of SEQ ID NO:5, a 3' miR155 scaffold sequence comprising the polynucleotide sequence of SEQ ID NO:41, a 3' arm of a rabbit β-globin intron comprising the polynucleotide sequence of SEQ ID NO:15, a minimal bovine growth hormone polyadenylation sequence comprising the polynucleotide sequence of SEQ ID NO:16, nucleic acid encoding a stuffer nucleic acid sequence from the human serpinA1 gene comprising the polynucleotide sequence of SEQ ID NO:19, and an AAV2 ITR comprising the polynucleotide sequence of SEQ ID NO:49; and wherein the capsid is an AAVrh74 W505R capsid. In some embodiments, the AAVrh74 W505R capsid comprises capsid proteins comprising the amino acid sequence of SEQ ID NO:52.

[0016] In some aspects, the invention provides a composition comprising any of the viral particles or rAAV particles described herein. In some embodiments, the invention provides a pharmaceutical composition comprising any of the viral particles or rAAV particles described herein. In some embodiments, the composition further comprises a pharmaceutically acceptable carrier.

[0017] In some aspects, the invention provides a modified desmin promoter (*e.g.*, for expression of a transgene in a muscle cell), wherein the modified desmin promoter comprises one or more enhancer elements and the promoter for the human desmin gene. In some embodiments, the modified desmin promoter comprises two enhancer elements and the promoter for the human desmin gene. In some embodiments, the modified desmin promoter comprises one or more Byrne enhancer elements and/or one or more Paulin enhancer elements. In some embodiments, the modified desmin promoter comprises one or more enhancer element comprising the nucleotide sequence of SEQ ID NO:21 or a sequence with about 90% identity to the sequence of SEQ ID NO:21 and/or one or more enhancer element comprising the nucleotide sequence of SEQ ID NO:22 or a sequence with about 90% identity to the sequence of SEQ ID NO:22. In some embodiments, the modified desmin promoter comprises the nucleotide sequence of SEQ ID NO:12 or a sequence with about 90% identity to the sequence of SEQ ID NO:12.

[0018] In some aspects, the invention provides kits comprising one or more of an RNAi as described herein, a viral particle as described herein, an AAV particle as described herein, or a composition as described herein. In some embodiments, the kit further comprises instructions for use.

[0019] In some aspects, the invention provides methods for treating myotonic dystrophy-1 (DM1) in a mammal in need thereof comprising administering to the mammal an effective amount of any of the RNAi described herein. In some aspects, the invention provides methods for inhibiting the expression of dystrophia myotonica protein kinase (DMPK) in a mammal with DM-1 in need thereof comprising administering to the mammal an effective amount of any of the RNAi described herein. In some aspects, the invention provides methods for inhibiting the accumulation of DMPK RNA in a cell of a mammal with DM-1 in need thereof comprising administering to the mammal an effective amount of any of the RNAi described herein.

[0020] In some aspects, the invention provides methods for treating myotonic dystrophy-1 (DM1) in a mammal in need thereof comprising administering to the mammal an effective amount of any of the viral particles (*e.g.*, rAAV particles) as described herein. In some aspects, the invention provides methods for inhibiting the expression of dystrophia myotonica protein

kinase (DMPK) in a mammal with DM-1 in need thereof comprising administering to the mammal an effective amount of any of the viral particles (*e.g.*, rAAV particles) as described herein. In some aspects, the invention provides methods for inhibiting the accumulation of DMPK RNA in a cell of a mammal with DM-1 in need thereof comprising administering to the mammal an effective amount of any of the viral particles (*e.g.*, rAAV particles) as described herein.

[0021] In some embodiments of the invention, the effective amount of the viral particles (*e.g.*, rAAV particles) is a dose of about 1 x 10^{8} to about 2 x 10^{13} genome copies/mL. In some embodiments of the invention, the dose is about 5 x 10^{12} genome copies/mL. In some embodiments of the invention, the dose is about 1 x 10^{13} genome copies/mL. In some embodiments of the invention, the dose is about 2 x 10^{13} genome copies/mL.

[0022] In some embodiments of the invention, the effective amount of the viral particles (*e.g.*, rAAV particles) is a dose of about 1 x 10^{8} to about 2 x 10^{14} genome copies/kg of body weight. In some embodiments of the invention, the dose is about 5 x 10^{13} genome copies/kg of body weight. In some embodiments of the invention, the dose is about 1 x 10^{14} genome copies/kg of body weight. In some embodiments of the invention, the dose is about 2 x 10^{14} genome copies/kg of body weight.

[0023] In some aspects, the invention provides methods for treating myotonic dystrophy-1 (DM1) in a mammal in need thereof comprising administering to the mammal an effective amount of any of the compositions as described herein. In some aspects, the invention provides methods for inhibiting the expression of dystrophia myotonica protein kinase (DMPK) in a mammal with DM-1 in need thereof comprising administering to the mammal an effective amount of a the composition of any of the composition as described herein. In some aspects, the invention provides methods for inhibiting the accumulation of DMPK RNA in a cell of a mammal with DM-1 in need thereof comprising administering to the mammal an effective amount of any of the composition as described herein.

[0024] In some embodiments of the invention, the RNAi is administered in combination with an immunosuppressive agent, wherein the immunosuppressive agent is administered before, at the same time, and/or after administration of the RNAi. In some embodiments, the viral particle or the rAAV particle is administered in combination with an immunosuppressive agent, wherein the immunosuppressive agent is administered before, at the same time, and/or after administration of the viral particle or the rAAV particle. In some embodiments, the composition is administered in combination with an immunosuppressive agent, wherein the

immunosuppressive agent is administered before, at the same time, and/or after administration of the composition.

DESCRIPTION OF THE DRAWINGS

[0025] The present application can be understood by reference to the following description taken in conjunction with the accompanying figures.

[0026] **FIG. 1A** depicts a sequence schematic of nDes-miR155- amiR-DMPK^{204} gene cassette. A hybrid muscle promoter is located upstream of the miR155- amiR-DMPK^{204} sequence. Downstream from the miRNA is a bovine growth hormone polyadenylation sequence (minBGHpA). A stuffer sequence from the A1AT intron flanks either side of the cassette. All of these are flanked by two AAV2 ITRs generating a combined vector genome size of 3739 bp.

[0027] **FIG. 1B** depicts the ITR plasmid used for cloning of the nDes-miR155- amiR-DMPK^{204} gene cassette. The ITR plasmid contains A1AT stuffer sequence flanked by the AAV2 5' and 3' ITRs. The A1AT stuffer sequence comprises NcoI and SphI restriction sites for cloning.

[0028] **FIG. 1C** depicts the results of a small-scale packaging assay was performed in HEK 293 cells to confirm packaging of the DC969-nDes-miR155- amiR-DMPK^{204} plasmid. Small-scale production was performed using the AAV rep/cap plasmid. The y-axis shows the amount of vector produced per HEK 293 cell as compared to a standard EGFP plasmid gene cassette (CD627-CBA-GFP). DRP: DNase resistance particle.

[0029] **FIGS. 2A-2C** depict an evaluation of DMPK knockdown by AAV the nDes-miR155-amiR-DMPK^{204} (amiR155-204) expression cassette in DMSXL mouse after tail vein injection. **FIG. 2A** shows transduction efficiency and biodistribution of AAV as evaluated by quantifying transgene copy numbers in the different organs. The qPCR results are expressed as mean ratio of AAV copy number/nuclei. **FIG. 2B** shows levels of amiR-DMPK^{204} in transduced tissues. MicroRNA input levels were normalized to U6 small nuclear RNA and set relative to BSS (Balance Salt Solution)-treated cells. **FIG. 2C** shows silencing of DMPK in transduced tissues. Total DMPK was determined by qRT-PCR. mRNA input was normalized to tata-box-binding protein (TBP) and set relative to BSS-treated cells. The dotted line indicates 50% DMPK expression relative to TBP expression. For **FIGS. 2A-2C**, data were evaluated using Student's T test, paired: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$. n= 13 (BSS), n=12 (amiR155-204).

[0030] **FIGS. 3A-3B** depict suppression of human DMPK by AAV nDes-miR155- amiR-DMPK^{204}. DMSXL mice were injected systemically with AAV nDes-miR155- amiR-DMPK^{204} in a dose dependent manner. The mice were euthanized after 8 weeks, organs were harvested, and amiR-DMPK^{204} and DMPK transcript levels were measured. **FIG. 3A** depicts the abundance of amiR-DMPK^{204} normalized to U6 in various tissues. **FIG. 3B** depicts the abundance of hDMPK transcripts normalized to mTBP in various tissues. Data were evaluated using ANOVA, multiple-comparison test: *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001. (n= wt-10, BSS- 7, low dose 5, medium dose 13 and high dose 7).

[0031] **FIG. 4** depicts correction of splicing abnormalities in DMSXL mice after systemic treatment with AAV nDes-miR155- amiR-DMPK^{204}. Splicing of alternative exon 11 in LDB3 was assessed using RT-PCR in gastrocnemius muscle after 8 weeks of treatment. Data were evaluated using ANOVA, multiple-comparison test: *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001. (n= wt-10, BSS- 7, low dose 5, medium dose 13 and high dose 7).

[0032] **FIGS. 5A** and **5B** show increased survival rate and body weight in female DMSXL mice treated with AAV nDes- miR155- amiR-DMPK^{204} in a doses dependent manner. **FIG. 5A** shows Kaplan-Meier survival curves showing improved survival rate with medium dose after 8 weeks of treatment as compared to low dose or BSS treated animals. **FIG. 5B** shows improved body weight observed in DMSXL animals treated with AAV nDes- miR155- amiR-DMPK^{204} as compared to BSS treated or low dose treated animals.

[0033] **FIG. 6** depicts reversal of electrophysiological features of DM1 disease upon systemic treatment of DMSXL DM1 mouse model with AAV nDes-miR155- amiR-DMPK^{204} in a dose dependent manner. Data were evaluated using ANOVA, multiple-comparison test: *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001. (n= wt-10, BSS- 7, low dose 5, medium dose 13 and high dose 7).

[0034] **FIG. 7A** shows a schematic representation of experimental protocol. Blood (🩸) was collected on day -1 to reconfirm the neutralizing antibodies. NHPs were administered with AAV encoding GFP reporter at a dose of 1e13vg/kg on day 0, and necropsy was performed on D21 and collected multiple tissues to evaluate the biodistribution. **FIGs.7B-7E** show quantification of GFP expression from various tissues of animals injected with AAV9, AAV rh74 and AAV rh74 N502I (rh74M). Bar graphs represents GFP quantities measured by ELISA in Tibialis anterior muscle (TA; **FIG. 7B**), Biceps Femoris (**FIG. 7C**), Quadriceps (**FIG. 7D**), Heart (**FIG. 7E**) and Liver (**FIG. 7F**). Data were evaluated using ANOVA, multiple-comparison test: *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.

[0035] **FIGs. 8A and 8B** show suppression of human DMPK by AAV rh74 N502I nDes-miR155- amiR-DMPK^{204}. DMSXL mice were injected systemically with AAV rh74 N502I nDes-miR155- amiR-DMPK^{204} in a dose dependent manner. The mice were euthanized after 8 weeks, organs were harvested, and amiR-DMPK^{204} and DMPK transcript levels were measured. **FIG. 8A** depicts the abundance of amiR-DMPK^{204} normalized to U6 in various tissues. **FIG. 8B** depicts the abundance of hDMPK transcripts normalized to mTBP in various tissues. Data were evaluated using ANOVA, multiple-comparison test: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$.

[0036] **FIG. 9** shows silencing of endogenous DMPK by AAVrh74N502I nDes-miR155-amiR-DMPK^{204}. Total DMPK levels were determined by qRT-PCR on RNA extracted from human DM1 cardiomyocytes differentiated from iPSCs. These cardiomyocytes were transduced with AAVrh74N502I nDes-miR155- amiR-DMPK^{204}. mRNA input levels were normalized to TBP mRNA. miRCTL3 served as a negative control and was set at 1. Student's T test, paired: **$p < 0.01$.

[0037] **FIG. 10** is a Volcano plot showing genome-wide gene expression changes in amiR-DMPK^{204} treated HEK293 cells, (Benjamini Hochberg FDR < 1%). Table showing top four differentially expressed (DE) genes with a 3' UTR seed complementary with FDR < 1% and FDR < 5%.

[0038] **FIG. 11** shows conservation of amiRDMPK^{204} target sequence among multiple sequences. Human target is SEQ ID NO:28, macaque target is SEQ ID NO:29, mouse target is SEQ ID NO:30, rat target is SEQ ID NO:31, and dog target is SEQ ID NO:32.

[0039] **FIG. 12** illustrates the biodistribution of viral genome copies/cell in multiple tissues in the indicated treatment groups.

[0040] **FIG. 13** shows the dose-dependent amiR-DMPK expression in various muscle tissues in the indicated treatment groups.

[0041] **FIG. 14** shows DMPK expression levels after treatment in various muscles for each indicated treatment group.

DETAILED DESCRIPTION

[0042] In some aspects, the invention provides an RNAi comprising a first strand and a second strand, wherein a) the first strand and the second strand form a duplex; b) the first strand comprises a guide region, wherein the guide region comprises nucleic acid with the sequence 5'-AGUCGAAGACAGUUCUAGGGU-3' (SEQ ID NO:1) or with a sequence with about 90%

identity to the sequence of SEQ ID NO:1; and c) the second strand comprises a non-guide region, wherein the non-guide region comprises nucleic acid with the sequence 5'-ACCCUAGAUGUCUUCGAUU-3' (SEQ ID NO:2) or a with a sequence with about 90% identity to the sequence of SEQ ID NO:2. In some embodiments, the invention provides expression cassettes for expressing nucleic acid encoding the RNAi; for example, for expressing the RNAi in muscles of a mammal. In some embodiments, the expression cassette is in an rAAV vector.

[0043] In some aspects, the invention provides methods for treating myotonic dystrophy 1 (DM-1) in a mammal by administering the RNAi of the invention to the mammal. In some embodiments, administered RNAi inhibits the expression of dystrophia myotonica protein kinase (DMPK) in the mammal; thereby ameliorating the DM-1 in the mammal.

General Techniques

[0044] The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in *Molecular Cloning: A Laboratory Manual* (Sambrook *et al.*, 4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2012); *Current Protocols in Molecular Biology* (F.M. Ausubel, *et al.* eds., 2003); the series *Methods in Enzymology* (Academic Press, Inc.); *PCR 2: A Practical Approach* (M.J. MacPherson, B.D. Hames and G.R. Taylor eds., 1995); *Antibodies, A Laboratory Manual* (Harlow and Lane, eds., 1988); *Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications* (R.I. Freshney, 6th ed., J. Wiley and Sons, 2010); *Oligonucleotide Synthesis* (M.J. Gait, ed., 1984); *Methods in Molecular Biology*, Humana Press; *Cell Biology: A Laboratory Notebook* (J.E. Cellis, ed., Academic Press, 1998); *Introduction to Cell and Tissue Culture* (J.P. Mather and P.E. Roberts, Plenum Press, 1998); *Cell and Tissue Culture: Laboratory Procedures* (A. Doyle, J.B. Griffiths, and D.G. Newell, eds., J. Wiley and Sons, 1993-8); *Handbook of Experimental Immunology* (D.M. Weir and C.C. Blackwell, eds., 1996); *Gene Transfer Vectors for Mammalian Cells* (J.M. Miller and M.P. Calos, eds., 1987); *PCR: The Polymerase Chain Reaction*, (Mullis *et al.*, eds., 1994); *Current Protocols in Immunology* (J.E. Coligan *et al.*, eds., 1991); *Short Protocols in Molecular Biology* (Ausubel *et al.*, eds., J. Wiley and Sons, 2002); *Immunobiology* (C.A. Janeway *et al.*, 2004); *Antibodies* (P. Finch, 1997); *Antibodies: A Practical Approach* (D. Catty., ed., IRL Press, 1988-1989); *Monoclonal Antibodies: A Practical Approach* (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); *Using Antibodies: A Laboratory Manual* (E. Harlow and D. Lane, Cold Spring Harbor

Laboratory Press, 1999); *The Antibodies* (M. Zanetti and J. D. Capra, eds., Harwood Academic Publishers, 1995); and *Cancer: Principles and Practice of Oncology* (V.T. DeVita *et al.*, eds., J.B. Lippincott Company, 2011).

Definitions

[0045] A "vector," as used herein, refers to a recombinant plasmid or virus that comprises a nucleic acid to be delivered into a host cell, either in vitro or in vivo.

[0046] The term "polynucleotide" or "nucleic acid" as used herein refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. Thus, this term includes, but is not limited to, single-, double- or multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, or a polymer comprising purine and pyrimidine bases, or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases. The backbone of the polynucleotide can comprise sugars and phosphate groups (as may typically be found in RNA or DNA), or modified or substituted sugar or phosphate groups. Alternatively, the backbone of the polynucleotide can comprise a polymer of synthetic subunits such as phosphoramidates and thus can be an oligodeoxynucleoside phosphoramidate (P-NH_2) or a mixed phosphoramidate- phosphodiester oligomer. In addition, a double-stranded polynucleotide can be obtained from the single stranded polynucleotide product of chemical synthesis either by synthesizing the complementary strand and annealing the strands under appropriate conditions, or by synthesizing the complementary strand de novo using a DNA polymerase with an appropriate primer.

[0047] The terms "polypeptide" and "protein" are used interchangeably to refer to a polymer of amino acid residues, and are not limited to a minimum length. Such polymers of amino acid residues may contain natural or non-natural amino acid residues, and include, but are not limited to, peptides, oligopeptides, dimers, trimers, and multimers of amino acid residues. Both full-length proteins and fragments thereof are encompassed by the definition. The terms also include post-expression modifications of the polypeptide, for example, glycosylation, sialylation, acetylation, phosphorylation, and the like. Furthermore, for purposes of the present invention, a "polypeptide" refers to a protein which includes modifications, such as deletions, additions, and substitutions (generally conservative in nature), to the native sequence, as long as the protein maintains the desired activity. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the proteins or errors due to PCR amplification.

[0048] A "recombinant viral vector" refers to a recombinant polynucleotide vector comprising one or more heterologous sequences (*i.e.*, nucleic acid sequence not of viral origin). In the case of recombinant AAV vectors, the recombinant nucleic acid is flanked by at least one, and in some embodiments two, inverted terminal repeat sequences (ITRs).

[0049] A "recombinant AAV vector (rAAV vector)" refers to a polynucleotide vector comprising one or more heterologous sequences (*i.e.*, nucleic acid sequence not of AAV origin) that are flanked by at least one, and in some embodiments two, AAV inverted terminal repeat sequences (ITRs). Such rAAV vectors can be replicated and packaged into infectious viral particles when present in a host cell that has been infected with a suitable helper virus (or that is expressing suitable helper functions) and that is expressing AAV rep and cap gene products (*i.e.* AAV Rep and Cap proteins). When a rAAV vector is incorporated into a larger polynucleotide (*e.g.*, in a chromosome or in another vector such as a plasmid used for cloning or transfection), then the rAAV vector may be referred to as a "pro-vector" which can be "rescued" by replication and encapsidation in the presence of AAV packaging functions and suitable helper functions. A rAAV vector can be in any of a number of forms, including, but not limited to, plasmids, linear artificial chromosomes, complexed with lipids, encapsulated within liposomes, and encapsidated in a viral particle, particularly an AAV particle. A rAAV vector can be packaged into an AAV virus capsid to generate a "recombinant adeno-associated viral particle (rAAV particle)".

[0050] "Heterologous" means derived from a genotypically distinct entity from that of the rest of the entity to which it is compared or into which it is introduced or incorporated. For example, a polynucleotide introduced by genetic engineering techniques into a different cell type is a heterologous polynucleotide (and, when expressed, can encode a heterologous polypeptide). Similarly, a cellular sequence (*e.g.*, a gene or portion thereof) that is incorporated into a viral vector is a heterologous nucleotide sequence with respect to the vector.

[0051] The term "transgene" refers to a polynucleotide that is introduced into a cell and is capable of being transcribed into RNA and optionally, translated and/or expressed under appropriate conditions. In aspects, it confers a desired property to a cell into which it was introduced, or otherwise leads to a desired therapeutic or diagnostic outcome. In another aspect, it may be transcribed into a molecule that mediates RNA interference, such as miRNA, siRNA, or shRNA.

[0052] The terms "genome particles (gp)," "genome equivalents," or "genome copies (gc)" as used in reference to a viral titer, refer to the number of virions containing the recombinant AAV DNA genome, regardless of infectivity or functionality. The number of genome particles

in a particular vector preparation can be measured by procedures such as described in the Examples herein, or for example, in Clark *et al.* (1999) *Hum. Gene Ther.*, 10:1031-1039; Veldwijk *et al.* (2002) *Mol. Ther.*, 6:272-278.

[0053] The term "vector genome (vg)" as used herein may refer to one or more polynucleotides comprising a set of the polynucleotide sequences of a vector, *e.g.*, a viral vector. A vector genome may be encapsidated in a viral particle. Depending on the particular viral vector, a vector genome may comprise single-stranded DNA, double-stranded DNA, or single-stranded RNA, or double-stranded RNA. A vector genome may include endogenous sequences associated with a particular viral vector and/or any heterologous sequences inserted into a particular viral vector through recombinant techniques. For example, a recombinant AAV vector genome may include at least one ITR sequence flanking a promoter, a stuffer, a sequence of interest (*e.g.*, an RNAi), and a polyadenylation sequence. A complete vector genome may include a complete set of the polynucleotide sequences of a vector. In some embodiments, the nucleic acid titer of a viral vector may be measured in terms of vg/mL. Methods suitable for measuring this titer are known in the art (*e.g.*, quantitative PCR).

[0054] As used herein, the term "inhibit" may refer to the act of blocking, reducing, eliminating, or otherwise antagonizing the presence, or an activity of, a particular target. Inhibition may refer to partial inhibition or complete inhibition. For example, inhibiting the expression of a gene may refer to any act leading to a blockade, reduction, elimination, or any other antagonism of expression of the gene, including reduction of mRNA abundance (*e.g.*, silencing mRNA transcription), degradation of mRNA, inhibition of mRNA translation, and so forth. In some embodiments, inhibiting the expression of DMPK may refer a blockade, reduction, elimination, or any other antagonism of expression of DMPK, including reduction of DMPK mRNA abundance (*e.g.*, silencing DMPK mRNA transcription), degradation of DMPK mRNA, inhibition of DMPK mRNA translation, and so forth. As another example, inhibiting the accumulation of a protein in a cell may refer to any act leading to a blockade, reduction, elimination, or other antagonism of expression of the protein, including reduction of mRNA abundance (*e.g.*, silencing mRNA transcription), degradation of mRNA, inhibition of mRNA translation, degradation of the protein, and so forth. In some embodiments, inhibiting the accumulation of DMPK protein in a cell refers to a blockade, reduction, elimination, or other antagonism of expression of the DMPK protein in a cell, including reduction of DMPK mRNA abundance (*e.g.*, silencing DMPK mRNA transcription), degradation of DMPK mRNA, inhibition of DMPK mRNA translation, degradation of the DMPK protein, and so forth

[0055] The terms "infection unit (iu)," "infectious particle," or "replication unit," as used in reference to a viral titer, refer to the number of infectious and replication-competent recombinant AAV vector particles as measured by the infectious center assay, also known as replication center assay, as described, for example, in McLaughlin *et al.* (1988) *J. Virol.*, 62:1963-1973.

[0056] The term "transducing unit (tu)" as used in reference to a viral titer, refers to the number of infectious recombinant AAV vector particles that result in the production of a functional transgene product as measured in functional assays such as described in Examples herein, or for example, in Xiao *et al.* (1997) *Exp. Neurobiol.*, 144:113-124; or in Fisher *et al.* (1996) *J. Virol.*, 70:520-532.

[0057] An "inverted terminal repeat" or "ITR" sequence is a term well understood in the art and refers to relatively short sequences found at the termini of viral genomes which are in opposite orientation.

[0058] An "AAV inverted terminal repeat (ITR)" sequence, a term well-understood in the art, is an approximately 145-nucleotide sequence that is present at both termini of the native single-stranded AAV genome. The outermost 125 nucleotides of the ITR can be present in either of two alternative orientations, leading to heterogeneity between different AAV genomes and between the two ends of a single AAV genome. The outermost 125 nucleotides also contains several shorter regions of self-complementarity (designated A, A', B, B', C, C' and D regions), allowing intrastrand base-pairing to occur within this portion of the ITR.

[0059] A "terminal resolution sequence" or "trs" is a sequence in the D region of the AAV ITR that is cleaved by AAV rep proteins during viral DNA replication. A mutant terminal resolution sequence is refractory to cleavage by AAV rep proteins.

[0060] "AAV helper functions" refer to functions that allow AAV to be replicated and packaged by a host cell. AAV helper functions can be provided in any of a number of forms, including, but not limited to, helper virus or helper virus genes which aid in AAV replication and packaging. Other AAV helper functions are known in the art such as genotoxic agents.

[0061] A "helper virus" for AAV refers to a virus that allows AAV (which is a defective parvovirus) to be replicated and packaged by a host cell. A helper virus provides "helper functions" which allow for the replication of AAV. A number of such helper viruses have been identified, including adenoviruses, herpesviruses and, poxviruses such as vaccinia and baculovirus. The adenoviruses encompass a number of different subgroups, although Adenovirus type 5 of subgroup C (Ad5) is most commonly used. Numerous adenoviruses of human, non-human mammalian and avian origin are known and are available from depositories such as the

ATCC. Viruses of the herpes family, which are also available from depositories such as ATCC, include, for example, herpes simplex viruses (HSV), Epstein-Barr viruses (EBV), cytomegaloviruses (CMV) and pseudorabies viruses (PRV). Examples of adenovirus helper functions for the replication of AAV include E1A functions, E1B functions, E2A functions, VA functions and E4orf6 functions. Baculoviruses available from depositories include Autographa californica nuclear polyhedrosis virus.

[0062] A preparation of rAAV is said to be "substantially free" of helper virus if the ratio of infectious AAV particles to infectious helper virus particles is at least about 102:l; at least about 104:l, at least about 106:l; or at least about 108:l or more. In some embodiments, preparations are also free of equivalent amounts of helper virus proteins (*i.e.*, proteins as would be present as a result of such a level of helper virus if the helper virus particle impurities noted above were present in disrupted form). Viral and/or cellular protein contamination can generally be observed as the presence of Coomassie staining bands on SDS gels (*e.g.*, the appearance of bands other than those corresponding to the AAV capsid proteins VPl, VP2 and VP3).

[0063] "Percent (%) sequence identity" with respect to a reference polypeptide or nucleic acid sequence is defined as the percentage of amino acid residues or nucleotides in a candidate sequence that are identical with the amino acid residues or nucleotides in the reference polypeptide or nucleic acid sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid or nucleic acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software programs, for example, those described in Current Protocols in Molecular Biology (Ausubel *et al.*, eds., 1987), Supp. 30, section 7.7.18, Table 7.7.1, and including BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. A preferred alignment program is ALIGN Plus (Scientific and Educational Software, Pennsylvania). Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows: 100 times the fraction X/Y, where X is the number of amino acid residues scored as identical matches by the sequence alignment program in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It

will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. For purposes herein, the % nucleic acid sequence identity of a given nucleic acid sequence C to, with, or against a given nucleic acid sequence D (which can alternatively be phrased as a given nucleic acid sequence C that has or comprises a certain % nucleic acid sequence identity to, with, or against a given nucleic acid sequence D) is calculated as follows: 100 times the fraction W/Z, where W is the number of nucleotides scored as identical matches by the sequence alignment program in that program's alignment of C and D, and where Z is the total number of nucleotides in D. It will be appreciated that where the length of nucleic acid sequence C is not equal to the length of nucleic acid sequence D, the % nucleic acid sequence identity of C to D will not equal the % nucleic acid sequence identity of D to C.

[0064] An "isolated" molecule (*e.g.*, nucleic acid or protein) or cell means it has been identified and separated and/or recovered from a component of its natural environment.

[0065] An "effective amount" is an amount sufficient to effect beneficial or desired results, including clinical results (*e.g.*, amelioration of symptoms, achievement of clinical endpoints, and the like). An effective amount can be administered in one or more administrations. In terms of a disease state, an effective amount is an amount sufficient to ameliorate, stabilize, or delay development of a disease.

[0066] An "individual" or "subject" is a mammal. Mammals include, but are not limited to, domesticated animals (*e.g.*, cows, sheep, cats, dogs, and horses), primates (*e.g.*, humans and non-human primates such as monkeys), rabbits, and rodents (*e.g.*, mice and rats). In certain embodiments, the individual or subject is a human.

[0067] As used herein, "treatment" is an approach for obtaining beneficial or desired clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (*e.g.*, not worsening) state of disease, preventing spread (*e.g.*, metastasis) of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment.

[0068] As used herein, the term "prophylactic treatment" refers to treatment, wherein an individual is known or suspected to have or be at risk for having a disorder but has displayed no symptoms or minimal symptoms of the disorder. An individual undergoing prophylactic treatment may be treated prior to onset of symptoms.

[0069] As used herein, the term "myotonic dystrophy type 1" or "DM1" refers to the a multisystem disorder that affects skeletal and smooth muscle as well as the eye, heart, endocrine system, and central nervous system. There are three overlapping categories of DM-1 (Bird, TD, *Myotonic Dystrophy Type 1*. 1999 Sep 17 [Updated 2021 Mar 25]. In: Adam MP, Ardinger HH, Pagon RA, *et al*., editors. *GeneReviews®* [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2022). Mild DM1 is characterized by cataract and mild myotonia, Classic DM1 is characterized by muscle weakness and wasting, myotonia, cataract, and often cardiac conduction abnormalities Congenital DM1 is characterized by hypotonia and severe generalized weakness at birth, often with respiratory insufficiency and early death; intellectual disability is common.

[0070] As used herein, the term "dystrophia myotonica protein kinase", "DMPK" "myotonin-protein kinase", "MT-PK", "myotonic dystrophy protein kinase" or "MDPK" may refer either to the gene or to a polypeptide product thereof associated with most cases of DM1. The 3' untranslated region of the DMPK gene contains 5-37 copies of a CTG trinucleotide repeat. Expansion of this unstable motif to 50-1,000 copies causes myotonic dystrophy type I, which increases in severity with increasing repeat element copy number.

[0071] As used herein, an "RNAi" may refer to any RNA molecule that induces RNA interference in a cell. Examples of RNAi include without limitation small inhibitory RNAs (siRNAs), microRNAs (miRNAs), and small hairpin RNAs (shRNAs).

[0072] An "miRNA" may refer to a polynucleotide containing (i) a double-stranded sequence targeting a gene of interest for knockdown by RNAi and (ii) additional sequences that form a stem-loop structure resembling that of endogenous miRNAs. In some embodiments, the miRNA includes nucleic acid flanking the stem-loop structure. These flanking sequences are knows as the "miRNA scaffold." A sequence targeting a gene of interest for RNAi (*e.g.*, a short, ~20-nt sequence) may be ligated to sequences that create a miRNA-like stem-loop and a sequence that base pairs with the sequence of interest to form a duplex when the polynucleotide is assembled into the miRNA-like secondary structure. As described herein, this duplex may hybridize imperfectly, *e.g.*, it may contain one or more unpaired or mispaired bases. Upon cleavage of this polynucleotide by Dicer, this duplex containing the sequence targeting a gene of interest may be unwound and incorporated into the RISC complex. A miRNA scaffold may refer to the miRNA itself or to a DNA polynucleotide encoding the miRNA. Examples of a miRNA scaffold include the miR-155 sequence (Lagos-Quintana, M. *et al*. (2002) *Curr. Biol*. 12:735-9) and the mirGE scaffold (WO2014016817A2). Commercially available kits for cloning a sequence into a

miRNA scaffold are known in the art (*e.g.*, the Invitrogen™ BLOCK-iT™ Pol II miR RNAi expression vector kit from Life Technologies, Thermo Fisher Scientific; Waltham, MA).

[0073] As used herein, the term "sense" nucleic acid is a nucleic acid comprising a sequence that encodes all or a part of a transgene. In some examples, mRNA for a transgene is a sense nucleic acid.

[0074] As used herein, "antisense" nucleic acid is a sequence of nucleic acid that is complementary to a "sense" nucleic acid. For example, an antisense nucleic acid may be complementary to a mRNA encoding a transgene.

[0075] As used herein, the "guide region" of an RNAi is the strand of the RNAi that binds the target mRNA, typically on the basis of complementarity. The region of complementarity may encompass the all or a portion of the guide region. Typically, the region of complementarity includes at least the seed region. In many cases, the antisense region of a RNAi is the guide region.

[0076] As used herein, the "passenger region," or "non-guide region," used interchangeably herein, of an RNAi is the region of the RNAi that is complementary to the guide region. In many cases, the sense region of a RNAi is the passenger region.

[0077] As used herein, the "seed region" of a RNAi (*e.g.*, miRNA) is a region of about 1-8 nucleotides in length of a microRNA. In some examples, the seed region and the 3'-UTR of its target mRNA may be a key determinant in RNAi recognition.

[0078] As used herein, "off-target gene silencing" refers to the pairing of a seed region of an RNAi with sequences in 3?-UTRs of unintended mRNAs and directs translational repression and destabilization of those transcripts (*e.g.*, reduces expression of the unintended mRNAs).

[0079] Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X."

[0080] As used herein, the singular form of the articles "a," "an," and "the" includes plural references unless indicated otherwise.

[0081] It is understood that aspects and embodiments of the invention described herein include "comprising," "consisting," and/or "consisting essentially of" aspects and embodiments.

RNAi

[0082] In some aspects, the invention provides improved RNAi targeting DMPK RNA for the treatment of myotonic dystrophy type 1 (DM1). In some embodiments, the RNAi is a small inhibitory RNA (siRNA), a microRNA (miRNA), or a small hairpin RNA (shRNA). A small inhibitory or interfering RNA (siRNA) is known in the art as a double-stranded RNA molecule of approximately 19-25 (*e.g.*, 19-23) base pairs in length that induces RNAi in a cell. miRNAs are typically smaller than siRNAs, can have multiple targets, and function to repress translation, degrade mRNA and in some instances cleaves mRNA endonucleolytically. A small hairpin RNA (shRNA) is known in the art as an RNA molecule comprising approximately 19-25 (*e.g.*, 19-23) base pairs of double stranded RNA linked by a short loop (*e.g.*, ~4-11 nucleotides) that induces RNAi in a cell. In some embodiments, the RNAi comprises a first strand and a second strand, wherein a) the first strand and the second strand form a duplex; b) the first strand comprises a guide region, wherein the guide region comprises the nucleic acid sequence 5'-AGUCGAAGACAGUUCUAGGGU-3' (SEQ ID NO:1); and c) the second strand comprises a non-guide region. In some embodiments, the nucleic the guide region comprises the nucleic acid sequence 5'-AGUCGAAGACAGUUCUAGGGU-3' (SEQ ID NO:1) and the non-guide region comprises the sequence 5'-ACCCUAGAUGUCUUCGAUU-3' (SEQ ID NO:2).

[0083] In some embodiments, the first strand comprises a guide region, wherein the guide region comprises a nucleic acid sequence having more than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to 5'-AGUCGAAGACAGUUCUAGGGU-3' (SEQ ID NO:1). In some embodiments, the first strand comprises a guide region, wherein the guide region comprises a nucleic acid sequence having more than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to 5'-AGUCGAAGACAGUUCUAGGGU-3' (SEQ ID NO:1) but maintains at least one CpG motif. In some embodiments, the second strand comprises a non-guide region, wherein the non-guide region comprises a nucleic acid sequence having more than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to 5'-ACCCUAGAUGUCUUCGAUU-3' (SEQ ID NO:2). In some embodiments, the second strand comprises a non-guide region, wherein the non-guide region comprises a nucleic acid sequence having more than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to 5'-ACCCUAGAUGUCUUCGAUU-3' (SEQ ID NO:2) but maintains at least one CpG motif.

[0084] In some embodiments, the RNAi comprises the nucleic acid sequence of SEQ ID NO:7. In some embodiments, the RNAi comprises a nucleic acid sequence having more than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to SEQ ID NO:7. In some

embodiments, the RNAi comprises a nucleic acid sequence having more than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to SEQ ID NO:7 but maintains at least one sequence (*e.g.*, in a seed sequence).

[0085] In some embodiments, the invention provides a nucleic acid encoding an RNAi comprises a first strand and a second strand, wherein a) the first strand and the second strand form a duplex; b) the first strand comprises a guide region, and c) the second strand comprises a non-guide region. In some embodiments, the nucleic acid encoding the RNAi comprises the nucleic acid sequence of SEQ ID NO:4 and/or the nucleic acid of SEQ ID NO:5. In some embodiments, the nucleic acid encoding the RNAi comprises a nucleic acid sequence having more than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to SEQ ID NO:4 and/or a nucleic acid sequence having more than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to SEQ ID NO:5. In some embodiments, the RNAi is encoded by the nucleic acid sequence of SEQ ID NO:8. In some embodiments, the RNAi is encoded by a nucleic acid sequence having more than about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to SEQ ID NO:8.

[0086] A microRNA (miRNA) is known in the art as an RNA molecule that induces RNAi in a cell comprising a short (*e.g.,* 19-25 base pairs) sequence of double-stranded RNA linked by a loop and containing one or more additional sequences of double-stranded RNA comprising one or more bulges (*e.g.,* mispaired or unpaired base pairs). As used herein, the term "miRNA" encompasses endogenous miRNAs as well as exogenous or heterologous miRNAs. In some embodiments, "miRNA" may refer to a pri-miRNA or a pre-miRNA. During miRNA processing, a pri-miRNA transcript is produced. The pri-miRNA is processed by Drosha-DGCR8 to produce a pre-miRNA by excising one or more sequences to leave a pre-miRNA with a 5'flanking region, a guide strand, a loop region, a non-guide strand, and a 3'flanking region; or a 5'flanking region, a non-guide strand, a loop region, a guide strand, and a 3'flanking region. The pre-miRNA is then exported to the cytoplasm and processed by Dicer to yield a siRNA with a guide strand and a non-guide (or passenger) strand. The guide strand is then used by the RISC complex to catalyze gene silencing, *e.g.,* by recognizing a target RNA sequence complementary to the guide strand. Further description of miRNAs may be found, *e.g.,* in WO 2008/150897. The recognition of a target sequence by a miRNA is primarily determined by pairing between the target and the miRNA seed sequence, *e.g.,* nucleotides 1-8 (5' to 3') of the guide strand (see, *e.g.,* Boudreau, R.L. *et al.* (2013) *Nucleic Acids Res.* 41:e9).

[0087] In the pri/pre-miRNA structure, the guide strand:non-guide strand interface in a duplex is formed in part through complementary base pairing (*e.g.,* Watson-Crick base pairing). However, in some embodiments, this complementary base pairing does not extend through the entire duplex. In some embodiments, a bulge in the interface may exist at one or more nucleotide positions. As used herein, the term "bulge" may refer to a region of nucleic acid that is non-complementary to the nucleic acid opposite it in a duplex. In some embodiments, the bulge is formed when the regions of complementary nucleic acids bind to each other, whereas the regions of central non-complementary region do not bind. In some embodiments, the bulge is formed when the two strands of nucleic acid positioned between the two complementary regions are of different lengths. As described below, a bulge may comprise 1 or more nucleotides.

[0088] During miRNA processing, the miRNA is cleaved at a cleavage site adjacent to the guide strand:non-guide strand interface, thus releasing the siRNA duplex of the guide and non-guide strands. In some embodiments, the miRNA comprises a bulge in the sense or antisense strand adjacent to the cleavage site. To state another way, in some embodiments, the miRNA comprises a bulge in the guide or non-guide strand adjacent to the seed sequence.

[0089] In some embodiments, the miRNA comprises a bulge in the guide strand opposite the 5' cleavage site of the mature non-guide strand. In some embodiments, the miRNA comprises a bulge opposite the 5' nucleotide of the non-guide strand. In some embodiments, the miRNA comprises a bulge in the sense strand opposite the 3' cleavage site of the mature guide strand. In some embodiments, the miRNA comprises a bulge opposite the 3' nucleotide of the guide strand.

[0090] The safety of RNAi-based therapies can be hampered by the ability of small inhibitory RNAs (siRNAs) to bind to unintended mRNAs and reduce their expression, an effect known as off-target gene silencing. Off-targeting primarily occurs when the seed region (nucleotides 2–8 of the small RNA) pairs with sequences in 3′-UTRs of unintended mRNAs and directs translational repression and destabilization of those transcripts. Reduced off-targeting RNAi may be designed by substituting bases within the guide and nonguide sequences; *e.g.*, by creating CpG motifs. Potential substitutions that may result in a significantly lower off-target score can be evaluated using the SiSPOTR algorithm, a specificity-focused siRNA design algorithm which identifies candidate sequences with minimal off-targeting potentials and potent silencing capacities (Boudreau *et al*, *Nucleic Acids Res*. 2013 Jan; 41(1) e9. A reduced SiSPOTR score predicts sequences that have a lower number of potential human off targets compared parent RNAi molecules. In some embodiments of the invention, the RNAi is improved to reduce off-target gene silencing.

[0091] In some embodiments, the first strand and the second strand are linked by means of a RNA (*e.g.*, a RNA linker) capable of forming a loop structure. As is commonly known in the art, an RNA loop structure (*e.g.,* a stem-loop or hairpin) is formed when an RNA molecule comprises two sequences of RNA that basepair together separated by a sequence of RNA that does not base pair together. For example, a loop structure may form in the RNA molecule A-B-C if sequences A and C are complementary or partially complementary such that they base pair together, but the bases in sequence B do not base pair together.

[0092] In some embodiments, the RNA capable of forming a loop structure comprises from 4 to 50 nucleotides. In certain embodiments, the RNA capable of forming a loop structure comprises 13 nucleotides. In some embodiments, the number of nucleotides in the RNA capable of forming a loop is from 4 to 50 nucleotides or any integer therebetween. In some embodiments, from 0-50% of the loop can be complementary to another portion of the loop. As used herein, the term "loop structure" is a sequence that joins two complementary strands of nucleic acid. In some embodiments, 1-3 nucleotides of the loop structure are contiguous to the complementary strands of nucleic acid and may be complementary to 1-3 nucleotides of the distal portion of the loop structure. For example, the three nucleotides at the 5' end of the loop structure may be complementary to the three nucleotides at the 3' end of the loop structure.

[0093] In some embodiments, nucleic acid encoding an RNAi of the present disclosure comprises a heterologous miRNA scaffold. In some embodiments, use of a heterologous miRNA scaffold is used to modulate miRNA expression; for example, to increase miRNA expression or to decrease miRNA expression. Any miRNA scaffold known in the art may be used. In some embodiments, the miRNA scaffold is derived from a miR-155 scaffold (see, *e.g.,* Lagos-Quintana, M. *et al.* (2002) *Curr. Biol.* 12:735-9 and the Invitrogen™ BLOCK-iT™ Pol II miR RNAi expression vector kit from Life Technologies, Thermo Fisher Scientific; Waltham, MA) or a mirGE scaffold (WO 2014/016817).

Methods to treat Myotonic Dystrophy Type-1 (DM-1)

[0094] Myotonic Dystrophy Type-1 (DM1) is a monogenic, autosomal-dominant, progressive disease caused by expansion of CTG repeats (>50) in the DMPK locus (dystrophia myotonica protein kinase). The DMPK with repeats are transcribed into mRNA, which forms hairpins and binds RNA binding proteins, sequestering them from their normal function. This leads to the appearance of nuclear foci, mis-splicing and ultimately myotonia. DM1 principally affects skeletal, cardiac and smooth muscle, resulting in significant physical, cognitive and behavioral impairments and disability.

[0095] In some aspects, the invention provides methods and compositions for treating myotonic dystrophy type 1 (DM1) in a mammal comprising administering to the mammal a pharmaceutical composition of the present disclosure (*e.g.*, a pharmaceutical composition comprising a rAAV particle of the present disclosure). In some aspects, the invention provides methods and compositions for inhibiting the expression of DMPK in a mammal with DM-1 comprising administering to the mammal a pharmaceutical composition of the present disclosure (*e.g.*, a pharmaceutical composition comprising a rAAV particle of the present disclosure). In some aspects, the invention provides methods and compositions for inhibiting the accumulation of DMPK in a cell of a mammal with DM1 comprising administering to the mammal a pharmaceutical composition of the present disclosure (*e.g.*, a pharmaceutical composition comprising a rAAV particle of the present disclosure). In some aspects, the invention provides methods and compositions for ameliorating a symptom of DM1, comprising administration of an effective amount of rAAV particles comprising a vector encoding an RNAi of the present disclosure to the muscle brain of a mammal.

[0096] In some aspects, the invention provides an RNAi for targeting DMPK mRNA in a mammal with DM1. In some embodiments, the RNAi comprises a first strand comprising a first nucleic acid comprising the sequence 5'- AGUCGAAGACAGUUCUAGGGU-3' (SEQ ID NO:1) and a second strand comprising a second nucleic acid comprising the sequence 5'-ACCCUAGAUGUCUUCGAUU-3' (SEQ ID NO:2). An RNAi described herein (*e.g.*, as part of a rAAV vector) may find use, inter alia, in treating DM1.

[0097] In some embodiments, the RNAi is a small inhibitory RNA (siRNA), a microRNA (miRNA), or a small hairpin RNA (shRNA). A small inhibitory or interfering RNA (siRNA) is known in the art as a double-stranded RNA molecule of approximately 19-25 (*e.g.*, 19-23) base pairs in length that induces RNAi in a cell. miRNAs are typically smaller than siRNAs, can have multiple targets, and function to repress translation, degrade mRNA and in some instances cleaves mRNA endonucleolytically. A small hairpin RNA (shRNA) is known in the art as an RNA molecule comprising approximately 19-25 (*e.g.*, 19-23) base pairs of double stranded RNA linked by a short loop (*e.g.*, ~4-11 nucleotides) that induces RNAi in a cell.

[0098] In some embodiments, the miRNA comprises a guide sequence that is about 90% identical to SEQ ID NO:1. In some embodiments, the miRNA comprises a guide sequence that is about any of 90% identical, 91% identical, 92% identical, 93% identical, 94% identical, 95% identical, 96% identical, 97% identical, 98% identical, 99% identical, or 100% identical to SEQ ID NO:1.

[0099] In some embodiments, the miRNA comprises a non-guide sequence (passenger strand) that is about 90% identical to SEQ ID NO:2. In some embodiments, the miRNA comprises a non-guide sequence that is about any of 90% identical, 91% identical, 92% identical, 93% identical, 94% identical, 95% identical, 96% identical, 97% identical, 98% identical, 99% identical, or 100% identical to SEQ ID NO:2.

[0100] In some embodiments, the first strand and the second strand are linked by means of RNA capable of forming a loop structure. As is commonly known in the art, an RNA loop structure (*e.g.*, a stem-loop or hairpin) is formed when an RNA molecule comprises two sequences of RNA that basepair together separated by a sequence of RNA that does not base pair together. For example, a loop structure may form in the RNA molecule A-B-C if sequences A and C are complementary or partially complementary such that they base pair together, but the bases in sequence B do not base pair together.

[0101] In some embodiments, the RNA capable of forming a loop structure comprises from 4 to 50 nucleotides. In certain embodiments, the RNA capable of forming a loop structure comprises 13 nucleotides. In certain embodiments, the RNA capable of forming a loop structure comprises the nucleotide sequence GUUUUGGCCACUGACUGAC (SEQ ID NO:3). In some embodiments, the vector genome comprises a nucleotide sequence that is at least about any of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO:3.

[0102] In some aspects, the invention provides methods comprising administering to a mammal (*e.g.*, a mammal with DM1) an RNAi comprising a first strand comprising a first nucleic acid comprising the sequence 5'- AGUCGAAGACAGUUCUAGGGU -3' (SEQ ID NO:1) and a second strand comprising a second nucleic acid comprising the sequence5'- ACCCUAGAUGUCUUCGAUU -3' (SEQ ID NO:2). In some embodiments, a recombinant viral particle comprises the RNAi. In some embodiments, the recombinant viral particle is an AAV particle encapsidating a rAAV vector, wherein the rAAV vector encodes the RNAi.

[0103] In some embodiments, delivery of rAAV particles is by systemic injection of rAAV particles to the mammal. In some embodiments, the systemic injection is intravenous injection, intra-arterial injection, intramuscular injection, intraperitoneal injection, intradermal injection, or subcutaneous injection, intra-CSF and intrathecal administrations (IT).

[0104] In some aspects, the invention provides methods for treating DM1 in a mammal comprising administering to the mammal the pharmaceutical composition of the present disclosure. In some aspects, the invention provides methods for inhibiting the accumulation of

DMPK in a cell of a mammal with DM1 comprising administering to the mammal the pharmaceutical composition of the present disclosure. In some aspects, the invention provides methods for inhibiting the expression of DMPK in a mammal with DM1 comprising administering to the mammal the pharmaceutical composition of the present disclosure. In some embodiments, the DMPK is a mutant DMPK (*e.g.*, an DMPK comprising greater than 37 or greater than 50 CTG repeats).

[0105] In some embodiments, the invention provides a method for treating a human with DM1 by administering an effective amount of a pharmaceutical composition comprising a rAAV vector encoding an RNAi of the present disclosure to suppress the activity of a mutant DMPK. In some embodiments, the pharmaceutical composition comprises one or more pharmaceutically acceptable excipients.

[0106] In some embodiments, the methods comprise administering an effective amount of a pharmaceutical composition comprising a rAAV vector encoding an RNAi of the present disclosure to suppress the activity of a mutant DMPK. In some embodiments, the viral titer of the rAAV particles is at least about any of 5×10^{12}, 6×10^{12}, 7×10^{12}, 8×10^{12}, 9×10^{12}, 10×10^{12}, 11×10^{12}, 15×10^{12}, 20×10^{12}, 25×10^{12}, 30×10^{12}, or 50×10^{12} genome copies/mL. In some embodiments, the viral titer of the rAAV particles is about any of 5×10^{12} to 6×10^{12}, 6×10^{12} to 7×10^{12}, 7×10^{12} to 8×10^{12}, 8×10^{12} to 9×10^{12}, 9×10^{12} to 10×10^{12}, 10×10^{12} to 11×10^{12}, 11×10^{12} to 15×10^{12}, 15×10^{12} to 20×10^{12}, 20×10^{12} to 25×10^{12}, 25×10^{12} to 30×10^{12}, 30×10^{12} to 50×10^{12} , or 50×10^{12} to 100×10^{12} genome copies/mL. In some embodiments, the viral titer of the rAAV particles is about any of 5×10^{12} to 10×10^{12}, 10×10^{12} to 25×10^{12}, or 25×10^{12} to 50×10^{12} genome copies/mL. In some embodiments, the viral titer of the rAAV particles is at least about any of 5×10^{9}, 6×10^{9}, 7×10^{9}, 8×10^{9}, 9×10^{9}, 10×10^{9}, 11×10^{9}, 15×10^{9}, 20×10^{9}, 25×10^{9}, 30×10^{9}, or 50×10^{9} transducing units /mL. In some embodiments, the viral titer of the rAAV particles is about any of 5×10^{9} to 6×10^{9}, 6×10^{9} to 7×10^{9}, 7×10^{9} to 8×10^{9}, 8×10^{9} to 9×10^{9}, 9×10^{9} to 10×10^{9}, 10×10^{9} to 11×10^{9}, 11×10^{9} to 15×10^{9}, 15×10^{9} to 20×10^{9}, 20×10^{9} to 25×10^{9}, 25×10^{9} to 30×10^{9}, 30×10^{9} to 50×10^{9} or 50×10^{9} to 100×10^{9} transducing units /mL. In some embodiments, the viral titer of the rAAV particles is about any of 5×10^{9} to 10×10^{9}, 10×10^{9} to 15×10^{9}, 15×10^{9} to 25×10^{9}, or 25×10^{9} to 50×10^{9} transducing units /mL. In some embodiments, the viral titer of the rAAV particles is at least any of about 5×10^{10}, 6×10^{10}, 7×10^{10}, 8×10^{10}, 9×10^{10}, 10×10^{10}, 11×10^{10}, 15×10^{10}, 20×10^{10}, 25×10^{10}, 30×10^{10}, 40×10^{10}, or 50×10^{10} infectious units/mL. In some embodiments, the viral titer of the rAAV particles is at least any of about 5×10^{10} to 6×10^{10}, 6×10^{10} to 7×10^{10}, 7×10^{10} to 8×10^{10}, 8×10^{10} to 9×10^{10}, 9×10^{10} to 10×10^{10}, 10×10^{10} to 11×10^{10}, 11×10^{10} to

15×10^{10}, 15×10^{10} to 20×10^{10}, 20×10^{10} to 25×10^{10}, 25×10^{10} to 30×10^{10}, 30×10^{10} to 40×10^{10}, 40×10^{10} to 50×10^{10}, or 50×10^{10} to 100×10^{10} infectious units/mL. In some embodiments, the viral titer of the rAAV particles is at least any of about 5×10^{10} to 10×10^{10}, 10×10^{10} to 15×10^{10}, 15×10^{10} to 25×10^{10}, or 25×10^{10} to 50×10^{10} infectious units/mL.

[0107] In some embodiments, the dose concentration of rAAV particles administered to the individual is any of about 1 x 10^8 to about 2 x 10^{13} genome copies/mL. In some embodiments, the dose concentration of rAAV particles administered to the individual is any of about 1 x 10^8 to about 5 x 10^8, about 5 x 10^8 to about 10 x 10^8, about 10 x 10^8 to about 20 x 10^8, about 20 x 10^8 to about 30 x 10^8, about 30 x 10^8 to about 40 x 10^8, about 40 x 10^8 to about 50 x 10^8, or about 50 x 10^8 to about 100 x 10^8 genome copies/mL. In some embodiments, the dose concentration of rAAV particles administered to the individual is any of about 1 x 10^9 to about 5 x 10^9, about 5 x 10^9 to about 10 x 10^9, about 10 x 10^9 to about 20 x 10^9, about 20 x 10^9 to about 30 x 10^9, about 30 x 10^9 to about 40 x 10^9, about 40 x 10^9 to about 50 x 10^9, or about 50 x 10^9 to about 100 x 10^9 genome copies/mL. In some embodiments, the dose concentration of rAAV particles administered to the individual is any of about 1 x 10^{10} to about 5 x 10^{10}, about 5 x 10^{10} to about 10 x 10^{10}, about 10 x 10^{10} to about 20 x 10^{10}, about 20 x 10^{10} to about 30 x 10^{10}, about 30 x 10^{10} to about 40 x 10^{10}, about 40 x 10^{10} to about 50 x 10^{10}, or about 50 x 10^{10} to about 100 x 10^{10} genome copies/mL. In some embodiments, the dose concentration of rAAV particles administered to the individual is any of about 1 x 10^{11} to about 5 x 10^{11}, about 5 x 10^{11} to about 10 x 10^{11}, about 10 x 10^{11} to about 20 x 10^{11}, about 20 x 10^{11} to about 30 x 10^{11}, about 30 x 10^{11} to about 40 x 10^{11}, about 40 x 10^{11} to about 50 x 10^{11}, or about 50 x 10^{11} to about 100 x 10^{11} genome copies/mL. In some embodiments, the dose concentration of rAAV particles administered to the individual is any of about 1 x 10^{12} to about 5 x 10^{12}, about 5 x 10^{12} to about 10 x 10^{12}, about 10 x 10^{12} to about 20 x 10^{12}, about 20 x 10^{12} to about 30 x 10^{12}, about 30 x 10^{12} to about 40 x 10^{12}, about 40 x 10^{12} to about 50 x 10^{12}, or about 50 x 10^{12} to about 100 x 10^{12} genome copies/mL. In some embodiments, the dose concentration of rAAV particles administered to the individual is any of about 1 x 10^{13} to about 2 x 10^{13} genome copies/mL. In some embodiments, the dose concentration of rAAV particles administered to the individual is about 1 x 10^8, about 5 x 10^8, about 1 x 10^9, about 5 x 10^9, about 1 x 10^{10}, about 5 x 10^{10}, about 1 x 10^{11}, about 5 x 10^{11}, about 1 x 10^{12}, about 5 x 10^{12}, about 1 x 10^{13}, or about 2 x 10^{13} genome copies/mL. In some embodiments, the dose concentration of rAAV particles administered to the individual is about 5 x 10^{12} genome copies/mL. In some embodiments, the dose concentration of rAAV particles administered to the individual is about 1 x 10^{13} genome copies/mL. In some

embodiments, the dose concentration of rAAV particles administered to the individual is about 2 x 10^{13} genome copies/mL.

[0108] In some embodiments, the dose of rAAV particles administered to the individual is at least about any of 1×10^8 to about 2×10^{14} genome copies/kg of body weight. In some embodiments, the dose of rAAV particles administered to the individual is between about any of 1×10^8 to about 2×10^{14} genome copies/kg of body weight. In some embodiments, the dose of rAAV particles administered to the individual is between any of about 1×10^8 to about 1×10^{14}, 5×10^8 to about 1×10^{14}, 1×10^9 to about 1×10^{14}, 5×10^9 to about 1×10^{14}, 1×10^{10} to about 1×10^{14}, 5×10^{10} to about 1×10^{14}, 1×10^{11} to about 1×10^{14}, 5×10^{11} to about 1×10^{14}, 1×10^{12} to about 1×10^{14}, 5×10^{12} to about 1×10^{14}, 1×10^{13} to about 1×10^{14}, 5×10^{13} to about 1×10^{14}, 1×10^8 to about 5×10^{13}, 5×10^8 to about 5×10^{13}, 1×10^9 to about 5×10^{13}, 5×10^9 to about 5×10^{13}, 1×10^{10} to about 5×10^{13}, 5×10^{10} to about 5×10^{13}, 1×10^{11} to about 5×10^{13}, 5×10^{11} to about 5×10^{13}, 1×10^{12} to about 5×10^{13}, 5×10^{12} to about 5×10^{13}, 1×10^{13} to about 5×10^{13}, 1×10^8 to about 1×10^{13}, 5×10^8 to about 1×10^{13}, 1×10^9 to about 1×10^{13}, 5×10^9 to about 1×10^{13}, 1×10^{10} to about 1×10^{13}, 5×10^{10} to about 1×10^{13}, 1×10^{11} to about 1×10^{13}, 5×10^{11} to about 1×10^{13}, 1×10^{12} to about 1×10^{13}, 5×10^{12} to about 1×10^{13}, 1×10^8 to about 5×10^{12}, 5×10^8 to about 5×10^{12}, 1×10^9 to about 5×10^{12}, 5×10^9 to about 5×10^{12}, 1×10^{10} to about 5×10^{12}, 5×10^{10} to about 5×10^{12}, 1×10^{11} to about 5×10^{12}, 5×10^{11} to about 5×10^{12}, 1×10^{12} to about 5×10^{12}, 1×10^8 to about 1×10^{12}, 5×10^8 to about 1×10^{12}, 1×10^9 to about 1×10^{12}, 5×10^9 to about 1×10^{12}, 1×10^{10} to about 1×10^{12}, 5×10^{10} to about 1×10^{12}, 1×10^{11} to about 1×10^{12}, 5×10^{11} to about 1×10^{12}, 1×10^8 to about 5×10^{11}, 5×10^8 to about 5×10^{11}, 1×10^9 to about 5×10^{11}, 5×10^9 to about 5×10^{11}, 1×10^{10} to about 5×10^{11}, 5×10^{10} to about 5×10^{11}, 1×10^{11} to about 5×10^{11}, 1×10^8 to about 1×10^{11}, 5×10^8 to about 1×10^{11}, 1×10^9 to about 1×10^{11}, 5×10^9 to about 1×10^{11}, 1×10^{10} to about 1×10^{11}, 5×10^{10} to about 1×10^{11}, 1×10^8 to about 5×10^{10}, 5×10^8 to about 5×10^{10}, 1×10^9 to about 5×10^{10}, 5×10^9 to about 5×10^{10}, 1×10^{10} to about 5×10^{10}, 1×10^8 to about 1×10^{10}, 5×10^8 to about 1×10^{10}, 1×10^9 to about 1×10^{10}, 5×10^9 to about 1×10^{10}, 1×10^8 to about 5×10^9, 5×10^8 to about 5×10^9, 1×10^9 to about 5×10^9, 1×10^8 to about 1×10^9, 5×10^8 to about 1×10^9, or 1×10^8 to about 5×10^8, gc/kg body weight. In some embodiments, the dose of rAAV particles administered to the individual is about 1 x 10^9, about 5 x 10^9, about 1 x 10^{10}, about 5 x 10^{10}, about 1 x 10^{11}, about 5 x 10^{11}, about 1 x 10^{12}, about 5 x 10^{12}, about 1 x 10^{13}, about 5 x 10^{13}, about 1 x 10^{14}, or about 2 x 10^{14} genome copies/kg body weight. In some embodiments, the dose of rAAV particles administered to the individual is about 5 x 10^{13} genome copies/kg body weight. In some embodiments, the dose of rAAV particles administered to the individual is about 1 x 10^{14} genome copies/kg body weight.

In some embodiments, the dose of rAAV particles administered to the individual is about 2 x 10^{14} genome copies/kg body weight.

[0109] In some embodiments, the total amount of rAAV particles administered to the individual is at least about any of 1×10^9 to about 2×10^{14} genome copies/kg body weight. In some embodiments, the total amount of rAAV particles administered to the individual is about any of 1×10^9 to about 2×10^{14} genome copies/kg body weight. In some embodiments of the invention, the volume of the composition injected to the striatum is more than about any one of 10 µl, 25 µl, 50 µl, 75 µl, 100 µl, 200 µl, 300 µl, 400 µl, 500 µl, 600 µl, 700 µl, 800 µl, 900 µl, 1 mL, 5 mL, 10 mL, 25 mL, 50 mL, 75 mL, or 100 mL or any amount therebetween.

[0110] Compositions of the invention (*e.g.*, rAAV particles comprising a vector encoding an RNAi of the present disclosure) can be used either alone or in combination with one or more additional therapeutic agents for treating DM1. The interval between sequential administration can be in terms of at least (or, alternatively, less than) minutes, hours, or days.

[0111] In some embodiments, the RNAi to treat DM1 is administered in combination with an immunosuppressive agent; for example, to suppress an immune response to the RNAi. In some embodiments, the immunosuppressive agent is administered before administration of the RNAi. In some embodiments, the immunosuppressive agent is administered at the same time as administration of the RNAi. In some embodiments, the immunosuppressive agent is administered after administration of the RNAi. In some embodiments, the immunosuppressive agent is administered in any combination of before, during or after administration of the RNAi.

[0112] In some embodiments, the rAAV particles to treat DM1 are administered in combination with an immunosuppressive agent; for example, to suppress an immune response to the rAAV particle and/or to the transgene product of the rAAV particle. In some embodiments, the immunosuppressive agent is administered before administration of the rAAV particle. In some embodiments, the immunosuppressive agent is administered at the same time as administration of the rAAV particle. In some embodiments, the immunosuppressive agent is administered after administration of the rAAV particle. In some embodiments, the immunosuppressive agent is administered in any combination of before, during or after administration of the rAAV particle.

[0113] In some embodiments, the invention provides the use of an effective amount of any of the RNAi described herein in the manufacture of a medicament for treating myotonic dystrophy-1 (DM1) in a mammal in need thereof. In some embodiments, the invention provides the use of

an effective amount of any of the RNAi described herein in the manufacture of a medicament for inhibiting the expression of dystrophia myotonica protein kinase (DMPK) in a mammal with DM-1 in need thereof. In some embodiments, the invention provides the use of an effective amount of any of the RNAi described herein in the manufacture of a medicament for inhibiting the accumulation of DMPK RNA in a cell of a mammal with DM-1 in need thereof.

[0114] In some embodiments, the invention provides the use of an effective amount of any of the RNAi described herein for treating myotonic dystrophy-1 (DM1) in a mammal in need thereof. In some embodiments, the invention provides the use of an effective amount of any of the RNAi described herein for inhibiting the expression of dystrophia myotonica protein kinase (DMPK) in a mammal with DM-1 in need thereof. In some embodiments, the invention provides the use of an effective amount of any of the RNAi described herein for inhibiting the accumulation of DMPK RNA in a cell of a mammal with DM-1 in need thereof.

[0115] In some embodiments, the invention provides an RNAi described herein for treating myotonic dystrophy-1 (DM1) in a mammal in need thereof. In some embodiments, the invention provides any of the RNAi described herein for inhibiting the expression of dystrophia myotonica protein kinase (DMPK) in a mammal with DM-1 in need thereof. In some embodiments, the invention provides any of the RNAi described herein for inhibiting the accumulation of DMPK RNA in a cell of a mammal with DM-1 in need thereof.

[0116] In some embodiments, the invention provides the use of an effective amount of any of the viral particles (*e.g.*, AAV particles) described herein in the manufacture of a medicament for treating myotonic dystrophy-1 (DM1) in a mammal in need thereof. In some embodiments, the invention provides the use of an effective amount of any of the viral particles (*e.g.*, AAV particles) described herein in the manufacture of a medicament for inhibiting the expression of dystrophia myotonica protein kinase (DMPK) in a mammal with DM-1 in need thereof. In some embodiments, the invention provides the use of an effective amount of any of the viral particles (*e.g.*, AAV particles) described herein in the manufacture of a medicament for inhibiting the accumulation of DMPK RNA in a cell of a mammal with DM-1 in need thereof.

[0117] In some embodiments, the invention provides the use of an effective amount of any of the viral particles (*e.g.*, AAV particles) described herein for treating myotonic dystrophy-1 (DM1) in a mammal in need thereof. In some embodiments, the invention provides the use of an effective amount of any of the viral particles (*e.g.*, AAV particles) described herein for inhibiting the expression of dystrophia myotonica protein kinase (DMPK) in a mammal with DM-1 in need thereof. In some embodiments, the invention provides the use of an effective amount of any of

the viral particles (*e.g.*, AAV particles) described herein for inhibiting the accumulation of DMPK RNA in a cell of a mammal with DM-1 in need thereof.

[0118] In some embodiments, the invention provides viral particles (*e.g.*, AAV particles) described herein for treating myotonic dystrophy-1 (DM1) in a mammal in need thereof. In some embodiments, the invention provides any of the viral particles (*e.g.*, AAV particles) described herein for inhibiting the expression of dystrophia myotonica protein kinase (DMPK) in a mammal with DM-1 in need thereof. In some embodiments, the invention provides any of the viral particles (*e.g.*, AAV particles) described herein for inhibiting the accumulation of DMPK RNA in a cell of a mammal with DM-1 in need thereof.

[0119] In some embodiments, the invention provides the use of an effective amount of any of the compositions described herein in the manufacture of a medicament for treating myotonic dystrophy-1 (DM1) in a mammal in need thereof. In some embodiments, the invention provides the use of an effective amount of any of the compositions described herein in the manufacture of a medicament for inhibiting the expression of dystrophia myotonica protein kinase (DMPK) in a mammal with DM-1 in need thereof. In some embodiments, the invention provides the use of an effective amount of any of the compositions described herein in the manufacture of a medicament for inhibiting the accumulation of DMPK RNA in a cell of a mammal with DM-1 in need thereof.

[0120] In some embodiments, the invention provides the use of an effective amount of any of the compositions described herein for treating myotonic dystrophy-1 (DM1) in a mammal in need thereof. In some embodiments, the invention provides the use of an effective amount of any of the compositions described herein for inhibiting the expression of dystrophia myotonica protein kinase (DMPK) in a mammal with DM-1 in need thereof. In some embodiments, the invention provides the use of an effective amount of any of the compositions described herein for inhibiting the accumulation of DMPK RNA in a cell of a mammal with DM-1 in need thereof.

[0121] In some embodiments, the invention provides compositions described herein for treating myotonic dystrophy-1 (DM1) in a mammal in need thereof. In some embodiments, the invention provides any of the compositions described herein for inhibiting the expression of dystrophia myotonica protein kinase (DMPK) in a mammal with DM-1 in need thereof. In some embodiments, the invention provides any of the compositions described herein for inhibiting the accumulation of DMPK RNA in a cell of a mammal with DM-1 in need thereof.

RNAi Expression Constructs and Vectors

[0122] The invention provides expression constructs, vectors and rAAV particles for expression of the RNAi described herein.

[0123] In some embodiments, nucleic acid encoding an RNAi of the present disclosure comprises a heterologous miRNA scaffold. In some embodiments, use of a heterologous miRNA scaffold is used to modulate miRNA expression; for example, to increase miRNA expression or to decrease miRNA expression. Any miRNA scaffold known in the art may be used. In some embodiments, the miRNA scaffold is derived from a miR-155 scaffold (see, *e.g.*, Lagos-Quintana, M. *et al.* (2002) *Curr. Biol.* 12:735-9 and the Invitrogen™ BLOCK-iT™ Pol II miR RNAi expression vector kit from Life Technologies, Thermo Fisher Scientific; Waltham, MA) or the mirGE scaffold (WO 2014/016817). In some embodiments, nucleic acid encoding an RNAi of the present disclosure comprises a miRNA scaffold. In some embodiments, miRNA scaffold is provided by SEQ ID NO:11. In some embodiments, the miRNA scaffold comprises a nucleic acid with greater than 80%, 85%, 90%, 95%, or 99% identity to the nucleic acid sequence of SEQ ID NO:11.

[0124] In some embodiments, the RNAi targets RNA encoding a polypeptide associated with DM1 (*e.g.*, mutant DMPK). Without wishing to be bound to theory, it is thought that an RNAi may be used to reduce or eliminate the expression and/or activity of a polypeptide whose gain-of-function has been associated with DM1 (*e.g.*, mutant DMPK).

[0125] In some embodiments, the transgene (*e.g.*, encoding an RNAi of the present disclosure) is operably linked to a promoter. Exemplary promoters include, but are not limited to, the cytomegalovirus (CMV) immediate early promoter, the RSV LTR, the MoMLV LTR, the phosphoglycerate kinase- 1 (PGK) promoter, a simian virus 40 (SV40) promoter and a CK6 promoter, a transthyretin promoter (TTR), a TK promoter, a tetracycline responsive promoter (TRE), an HBV promoter, an hAAT promoter, a LSP promoter, chimeric liver-specific promoters (LSPs), the E2F promoter, the telomerase (hTERT) promoter; the cytomegalovirus enhancer/chicken beta-actin/Rabbit β-globin promoter (CAG promoter; Niwa *et al.*, *Gene*, 1991, 108(2):193-9) and the elongation factor 1-alpha promoter (EFl-alpha) promoter (Kim *et al.*, *Gene*, 1990, 91(2):217-23 and Guo *et al.*, *Gene Ther.*, 1996, 3(9):802-10). In some embodiments, the promoter comprises a human β-glucuronidase promoter or a cytomegalovirus enhancer linked to a chicken β-actin (CBA) promoter. The promoter can be a constitutive, inducible or repressible promoter.

[0126] Inducible promoters allow regulation of gene expression and can be regulated by exogenously supplied compounds, environmental factors such as temperature, or the presence of a specific physiological state, *e.g.*, acute phase, a particular differentiation state of the cell, or in replicating cells only. Examples of inducible promoters regulated by exogenously supplied promoters include the zinc-inducible sheep metallothionine (MT) promoter, the dexamethasone (Dex)-inducible mouse mammary tumor virus (MMTV) promoter, the T7 polymerase promoter system (WO 98/10088); the ecdysone insect promoter (No *et al*, *Proc. Natl. Acad. Sci. USA*, 93:3346-3351 (1996)), the tetracycline-repressible system (Gossen *et al*, *Proc. Natl. Acad. Sci. USA*, 89:5547-5551 (1992)), the tetracycline-inducible system (Gossen *et al*, *Science*, 268:1766-1769 (1995), see also Harvey *et al*, *Curr. Opin. Chem. Biol.*, 2:512-518 (1998)), the RU486-inducible system (Wang *et al.*, *Nat. Biotech.*, 15:239-243 (1997) and Wang *et al.*, *Gene Ther.*, 4:432-441 (1997)) and the rapamycin-inducible system (Magari *et al.*, *J. Clin. Invest.*, 100:2865-2872 (1997)). Still other types of inducible promoters which may be useful in this context are those which are regulated by a specific physiological state, *e.g.*, temperature, acute phase, a particular differentiation state of the cell, or in replicating cells only.

[0127] In some embodiments, the regulatory sequences impart tissue-specific gene expression capabilities. In some cases, the tissue-specific regulatory sequences bind tissue-specific transcription factors that induce transcription in a tissue specific manner. Such tissue-specific regulatory sequences (*e.g.*, promoters, enhancers, *etc.*) are well known in the art. In some embodiments, the promoter is a muscle-specific promoter. In some embodiments, the promoter is a desmin promoter. In some embodiments, the promoter is a human desmin promoter (*e.g.*, −228 to +75 of the human desmin gene; *e.g.*, SEQ ID NO:23). In some embodiments, the promoter is a modified desmin promoter. In some embodiments, the desmin promoter comprises desmin promoter elements important for high level expression in muscle cells (Li and Paulin, *et. al*. 1991. *Journal of Biol Chem.*). In some embodiments, the desmin promoter comprises at least one copy of the Byrne desmin enhancer (*e.g.*, SEQ ID NO:21). In some embodiments, the desmin promoter comprises at least one copy of the Paulin desmin enhancer (-973 to -693) (*e.g.*, SEQ ID NO:22). In some embodiments, the desmin promoter comprises one copy of Byrne desmin enhance are one copy of the Paulin desmin enhancer (-973 to -693). In some embodiments, the desmin promoter comprises one copy of Byrne desmin enhance are one copy of the Paulin desmin enhancer (-973 to -693) and the promoter of the human desmin gene (−228 to +75).

[0128] In some aspects, the invention provides an expression cassette (*e.g.*, and expression cassette for expression of a transgene (*e.g.*, a therapeutic transgene) in a muscle cell), wherein the expression cassette comprises a modified desmin promoter, wherein the desmin promoter

comprises one or more enhancer elements and the promoter for the human desmin gene. In some embodiments, the desmin promoter comprises two enhancer elements and the promoter for the human desmin gene. In some embodiments, the desmin promoter comprises one or more Byrne enhancer element and/or one or more Paulin enhancer elements. In some embodiments, the desmin promoter comprises one or more enhancer element comprising the nucleotide sequence of SEQ ID NO:21, In some embodiments, the desmin promoter comprises one or more enhancer element comprising the nucleotide sequence of SEQ ID NO:22. In some embodiments, the desmin promoter comprises one or more enhancer element comprising the nucleotide sequence of SEQ ID NO:21 and one or more enhancer element comprising the nucleotide sequence of SEQ ID NO:22. In some embodiments, the desmin promoter comprises one or more enhancer element comprising a nucleotide sequence having at least about any of 80%, 85%, 90%, 95%, or 99% identity to the nucleotide sequence of SEQ ID NO:21. In some embodiments, the desmin promoter comprises one or more enhancer element comprising a nucleotide sequence having at least about any of 80%, 85%, 90%, 95%, or 99% identity to the nucleotide sequence of SEQ ID NO:22. In some embodiments, the desmin promoter comprises one or more enhancer element comprising a nucleotide sequence having at least about any of 80%, 85%, 90%, 95%, or 99% identity to the nucleotide sequence of SEQ ID NO:21 and one or more enhancer element comprising a nucleotide sequence having at least about any of 80%, 85%, 90%, 95%, or 99% identity to the nucleotide sequence of SEQ ID NO:22.

[0129] In some embodiments, the expression cassette comprising the modified desmin promoter further comprises an intron. In some embodiments, the intron is a rabbit β-globin intron. In some embodiments, the intron comprises the nucleotide sequence of SEQ ID NO:13. In some embodiments, the intron comprises a nucleotide sequence having at least about any of 80%, 85%, 90%, 95%, or 99% identity to the nucleotide sequence of SEQ ID NO:13. In some embodiments, the nucleic acid encoding the transgene (*e.g.*, a therapeutic transgene) is embedded in the intron. In some embodiments, the intron comprises a 5' arm and a 3' arm, wherein the 5' arm is located 5' to the nucleic acid encoding the transgene and the 3' arm is located 3' to the nucleic acid encoding the transgene. In some embodiments, the 5' arm of the intron comprises nucleic acid with the sequence of SEQ ID NO:14. In some embodiments, the 5' arm of the intron comprises nucleic acid with a sequence having at least about any of 80%, 85%, 90%, 95%, or 99% identity to the nucleotide sequence of SEQ ID NO:14. In some embodiments, the 3' arm of the intron comprises nucleic acid with the sequence of SEQ ID NO:15. In some embodiments, the 3' arm of the intron comprises nucleic acid with a sequence having at least about any of 80%, 85%, 90%, 95%, or 99% identity to the nucleotide sequence of SEQ ID NO:15. In some

embodiments, the 5' arm of the intron comprises nucleic acid with the sequence of SEQ ID NO:14 and the 3' arm of the intron comprises nucleic acid with the sequence of SEQ ID NO:15. In some embodiments, the 5' arm of the intron comprises nucleic acid with a sequence having at least about any of 80%, 85%, 90%, 95%, or 99% identity to the nucleotide sequence of SEQ ID NO:14 and the 3' arm of the intron comprises nucleic acid with a sequence having at least about any of 80%, 85%, 90%, 95%, or 99% identity to the nucleotide sequence of SEQ ID NO:15.

[0130] In some embodiments, the expression cassette comprising the modified desmin promoter further comprises a polyadenylation signal. In some embodiments, the polyadenylation signal is a bovine growth hormone polyadenylation signal, an SV40 polyadenylation signal, or a HSV TK pA. In some embodiments, the the polyadenylation signal is a minimal bovine growth hormone polyadenylation signal. In some embodiments, the bovine growth hormone polyadenylation signal comprises nucleic acid with the sequence of SEQ ID NO:16. In some embodiments, the bovine growth hormone polyadenylation signal comprises nucleic acid with a sequence having at least about any of 80%, 85%, 90%, 95%, or 99% identity to the nucleotide sequence of SEQ ID NO:16.

[0131] In some embodiments, the invention provides an expression cassette comprising a modified desmin promoter for expression of a transgene (*e.g.*, a therapeutic transgene) in a muscle cell. In some embodiments, the transgene encodes a polypeptide (*e.g.*, a therapeutic polypeptide). In some embodiments, the transgene encodes a nucleic acid (*e.g.*, a therapeutic nucleic acid). In some embodiments, the transgene encodes an RNAi. In some embodiments, the transgene encodes an siRNA, an shRNA, or an miRNA.

[0132] In some aspects, the invention provides a modified desmin promoter, wherein the desmin promoter comprises one or more enhancer elements and the promoter for the human desmin gene. In some embodiments, the desmin promoter comprises two enhancer elements and the promoter for the human desmin gene. In some embodiments, the desmin promoter comprises one or more Byrne enhancer element and/or one or more Paulin enhancer elements. In some embodiments, the desmin promoter comprises one or more enhancer element comprising the nucleotide sequence of SEQ ID NO:21, In some embodiments, the desmin promoter comprises one or more enhancer element comprising the nucleotide sequence of SEQ ID NO:22. In some embodiments, the desmin promoter comprises one or more enhancer element comprising the nucleotide sequence of SEQ ID NO:21 and one or more enhancer element comprising the nucleotide sequence of SEQ ID NO:22. In some embodiments, the desmin promoter comprises one or more enhancer element comprising a nucleotide sequence having at least about any of

80%, 85%, 90%, 95%, or 99% identity to the nucleotide sequence of SEQ ID NO:21. In some embodiments, the desmin promoter comprises one or more enhancer element comprising a nucleotide sequence having at least about any of 80%, 85%, 90%, 95%, or 99% identity to the nucleotide sequence of SEQ ID NO:22. In some embodiments, the desmin promoter comprises one or more enhancer element comprising a nucleotide sequence having at least about any of 80%, 85%, 90%, 95%, or 99% identity to the nucleotide sequence of SEQ ID NO:21 and one or more enhancer element comprising a nucleotide sequence having at least about any of 80%, 85%, 90%, 95%, or 99% identity to the nucleotide sequence of SEQ ID NO:22.

[0133] In some aspects, the invention provides rAAV particles comprising a recombinant self-complementing genome (*e.g.*, a self-complementary rAAV vector). AAV viral particles with self-complementing vector genomes and methods of use of self-complementing AAV genomes are described in US Patent Nos. 6,596,535; 7,125,717; 7,465,583; 7,785,888; 7,790,154; 7,846,729; 8,093,054; and 8,361,457; and Wang Z., *et al.*, (2003) *Gene Ther* 10:2105-2111, each of which are incorporated herein by reference in its entirety. A rAAV comprising a self-complementing genome will quickly form a double stranded DNA molecule by virtue of its partially complementing sequences (*e.g.*, complementing coding and non-coding strands of a heterologous nucleic acid). In some embodiments, the vector comprises first nucleic acid sequence encoding the heterologous nucleic acid and a second nucleic acid sequence encoding a complement of the nucleic acid, where the first nucleic acid sequence can form intrastrand base pairs with the second nucleic acid sequence along most or all of its length.

[0134] In some embodiments, the first heterologous nucleic acid sequence encoding a RNAi and a second heterologous nucleic acid sequence encoding the complement of the RNAi are linked by a mutated ITR (*e.g.*, the right ITR). In some embodiments, the ITR comprises the polynucleotide sequence 5'-CCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGA-3 (SEQ ID NO:27). The mutated ITR comprises a deletion of the D region comprising the terminal resolution sequence. As a result, on replicating an AAV viral genome, the rep proteins will not cleave the viral genome at the mutated ITR and as such, a recombinant viral genome comprising the following in 5' to 3' order will be packaged in a viral capsid: an AAV ITR, the first heterologous polynucleotide sequence including regulatory sequences, the mutated AAV ITR, the second heterologous polynucleotide in reverse orientation to the first heterologous polynucleotide and a third AAV ITR.

rAAV particles and methods of producing rAAV particles

[0135] The invention provides rAAV particles comprising the RNAi as disclosed herein. In some embodiments, the invention provides methods of using recombinant viral particles to deliver RNAi to treat a DM1. In some embodiments, the rAAV particle comprises a sequence encoding the RNAi of the present disclosure flanked by one or two ITRs. The nucleic acid is encapsidated in the AAV particle. The AAV particle also comprises capsid proteins. In some embodiments, the nucleic acid comprises the coding sequence(s) of interest (*e.g.*, nucleic acid encoding the RNAi of the present disclosure) operatively linked components in the direction of transcription, control sequences including transcription initiation and termination sequences, thereby forming an expression cassette. The expression cassette is flanked on the 5' and 3' end by at least one functional AAV ITR sequences. By "functional AAV ITR sequences" it is meant that the ITR sequences function as intended for the rescue, replication and packaging of the AAV virion. *See* Davidson *et al.*, *PNAS*, 2000, 97(7)3428-32; Passini *et al.*, *J. Virol.*, 2003, 77(12):7034-40; and Pechan *et al.*, *Gene Ther.*, 2009, 16:10-16, all of which are incorporated herein in their entirety by reference. For practicing some aspects of the invention, the recombinant vectors comprise at least all of the sequences of AAV essential for encapsidation and the physical structures for infection by the rAAV. AAV ITRs for use in the vectors of the invention need not have a wild-type nucleotide sequence (*e.g.*, as described in Kotin, *Hum. Gene Ther.*, 1994, 5:793-801), and may be altered by the insertion, deletion or substitution of nucleotides or the AAV ITRs may be derived from any of several AAV serotypes. More than 40 serotypes of AAV are currently known, and new serotypes and variants of existing serotypes continue to be identified. *See* Gao *et al.*, *PNAS*, 2002, 99(18): 11854-6; Gao *et al.*, *PNAS*, 2003, 100(10):6081-6; and Bossis *et al.*, *J. Virol.*, 2003, 77(12):6799-810. Use of any AAV serotype is considered within the scope of the present invention. In some embodiments, a rAAV vector is a vector derived from an AAV serotype, including without limitation, AAV ITRs are AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV2R471A, AAVrh74, AAV DJ, a goat AAV, bovine AAV, or mouse AAV capsid serotype ITRs or the like. In some embodiments, the nucleic acid in the AAV comprises an ITR of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAVrh74, AAV DJ, a goat AAV, bovine AAV, or mouse AAV capsid serotype ITRs or the like. In some embodiments, the nucleic acid in the AAV further encodes an RNAi as described herein. For example, the nucleic acid in the AAV can comprise at least one ITR of any AAV serotype contemplated herein and can further encode an RNAi comprising one strand that comprises a guide region and another

strand that comprises a non-guide region. In one embodiment, the nucleic acid in the AAV can comprise at least one ITR of any AAV serotype and can further encode an RNAi comprising a first strand comprising a first nucleic acid comprising the sequence 5'-AGUCGAAGACAGUUCUAGGGU -3' (SEQ ID NO:1) or a sequence with 80%, 85%, 90%, or 95% identity to SEQ ID NO:1, and a second strand comprising a second nucleic acid comprising the sequence 5'- ACCCUAGAUGUCUUCGAUU -3' (SEQ ID NO:2) or a sequence with 80%, 85%, 90%, or 95% identity to SEQ ID NO:2.

[0136] In some embodiments, the nucleic acid in the AAV comprises 5' to 3' nucleic acid encoding the following: an ITR (*e.g.*, an AAV2 ITR), a promoter, a nucleic acid encoding an RNAi as disclosed herein, a polyadenylation signal, and an AAV ITR (*e.g.*, an AAV2 ITR). In some embodiments, the nucleic acid in the AAV comprises 5' to 3' nucleic acid encoding the following: an ITR (*e.g.*, an AAV2 ITR), a promoter, a nucleic acid encoding an RNAi comprising a first strand comprising a first nucleic acid comprising the sequence 5'-AGUCGAAGACAGUUCUAGGGU -3' (SEQ ID NO:1) or a sequence with 80%, 85%, 90%, or 95% identity to SEQ ID NO:1, and a second strand comprising a second nucleic acid comprising the sequence 5'-ACCCUAGAUGUCUUCGAUU-3' (SEQ ID NO:2) or a sequence with 80%, 85%, 90%, or 95% identity to SEQ ID NO:2, a polyadenylation signal, and an AAV ITR (*e.g.*, an AAV2 ITR). In some embodiments, the nucleic acid in the AAV comprises 5' to 3' nucleic acid encoding the following: an ITR (*e.g.*, an AAV2 ITR), a desmin promoter, a nucleic acid encoding an RNAi as disclosed herein, a polyadenylation signal (*e.g.*, a bovine growth hormone polyA), and an AAV ITR (*e.g.*, an AAV2 ITR). In some embodiments, the nucleic acid in the AAV comprises 5' to 3' nucleic acid encoding the following: all or a functional portion of an ITR (*e.g.*, an AAV2 ITR), a desmin promoter, an intron (*e.g.*, a chimeric intron), a nucleic acid encoding an RNAi comprising a first strand comprising a first nucleic acid comprising the sequence 5'-AGUCGAAGACAGUUCUAGGGU-3' (SEQ ID NO:1) or a sequence with 80%, 85%, 90%, or 95% identity to SEQ ID NO:1, and a second strand comprising a second nucleic acid comprising the sequence 5'- ACCCUAGAUGUCUUCGAUU -3' (SEQ ID NO:2) or a sequence with 80%, 85%, 90%, or 95% identity to SEQ ID NO:2, a polyadenylation signal (*e.g.*, a bovine growth hormone polyA), and an AAV ITR (*e.g.*, an AAV2 ITR). In some embodiments, the first strand and second strand form a duplex. In some embodiments, the first strand is linked to the second strand by a linker. In some embodiments, the linker comprises the nucleic acid sequence of SEQ ID NO:3 or a sequence with 80%, 85%, 90%, or 95% identity to SEQ ID NO:3.

[0137] In some embodiments, the nucleic acid in the AAV comprises 5' to 3' nucleic acid encoding the following: all or a functional portion of an ITR (*e.g.*, an AAV2 ITR), a stuffer

sequence (*e.g.*, all or a portion of a human alpha-1-antitrypsin (AAT) stuffer sequence), a desmin promoter, a 5' arm of an intron (*e.g.*, a rabbit β-globin intron), a nucleic acid encoding an RNAi comprising a first strand comprising a first nucleic acid comprising the sequence 5'-AGUCGAAGACAGUUCUAGGGU-3' (SEQ ID NO:1) or a sequence with 80%, 85%, 90%, or 95% identity to SEQ ID NO:1, and a second strand comprising a second nucleic acid comprising the sequence 5'- ACCCUAGAUGUCUUCGAUU -3' (SEQ ID NO:2) or a sequence with 80%, 85%, 90%, or 95% identity to SEQ ID NO:2, a 3' arm of an intron (*e.g.*, a rabbit β-globin intron), a polyadenylation signal (*e.g.*, a bovine growth hormone polyA),), a stuffer sequence (*e.g.*, all or a portion of a human alpha-1-antitrypsin (AAT) stuffer sequence) and an AAV ITR (*e.g.*, an AAV2 ITR).

[0138] In some embodiments, the nucleic acid in the AAV comprises 5' to 3' nucleic acid encoding the following: an ITR (*e.g.*, an AAV2 ITR), a desmin promoter, a nucleic acid encoding an RNAi comprising a first strand comprising a first nucleic acid comprising the sequence 5'-ACCCUAGAUGUCUUCGAUU -3' (SEQ ID NO:2) or a sequence with 80%, 85%, 90%, or 95% identity to SEQ ID NO:2, and a second strand comprising a second nucleic acid comprising the sequence 5'- ACCCUAGAUGUCUUCGAUU -3' (SEQ ID NO:1) or a sequence with 80%, 85%, 90%, or 95% identity to SEQ ID NO:1, a polyadenylation signal (*e.g.*, a bovine growth hormone polyA), and an AAV ITR (*e.g.*, an AAV2 ITR). In some embodiments, the first strand and second strand form a duplex. In some embodiments, the first strand is linked to the second strand by a linker. In some embodiments, the linker comprises the nucleic acid sequence of SEQ ID NO:6.

[0139] In some embodiments, the nucleic acid in the AAV comprises 5' to 3' nucleic acid encoding the following: all or a functional portion of an ITR (*e.g.*, an AAV2 ITR), a stuffer sequence (*e.g.*, all or a portion of a human alpha-1-antitrypsin (AAT) stuffer sequence), a desmin promoter, a 5' arm of an intron (*e.g.*, a rabbit β-globin intron), a nucleic acid encoding an RNAi comprising a first strand comprising a first nucleic acid comprising the sequence 5'-ACCCUAGAUGUCUUCGAUU -3' (SEQ ID NO:2), and a second strand comprising a second nucleic acid comprising the sequence 5'-AGUCGAAGACAGUUCUAGGGU-3' (SEQ ID NO:1), a 3' arm of an intron (*e.g.*, a rabbit β-globin intron), a polyadenylation signal (*e.g.*, a bovine growth hormone polyA),), a stuffer sequence (*e.g.*, all or a portion of a human alpha-1-antitrypsin (AAT) stuffer sequence) and an AAV ITR (*e.g.*, an AAV2 ITR).

[0140] In some embodiments, a vector may include a (one or more) stuffer nucleic acid. In some embodiments, the stuffer nucleic acid may comprise a sequence that encodes a reporter

polypeptide. As will be appreciated by those of skill in the art, the stuffer nucleic acid may be located in a variety of regions within the vector, and may be comprised of a continuous sequence (*e.g.*, a single stuffer nucleic acid in a single location) or multiple sequences (*e.g.*, more than one stuffer nucleic acid in more than one location (*e.g.*, 2 locations, 3 locations, *etc.*) within the vector. In some embodiments, the stuffer nucleic acid may be located downstream of the RNAi sequence. In embodiments, the stuffer nucleic acid may be located upstream of the RNAi sequence (*e.g.*, between the promoter and the nucleic acid encoding the RNAi). As will also be appreciated by those of skill in the art a variety of nucleic acids may be used as a stuffer nucleic acid. In some embodiments, the stuffer nucleic acid comprises all or a portion of a human alpha-1-antitrypsin (AAT) stuffer sequence or a C16 P1 chromosome 16 P1 clone (human C16) stuffer sequence. In some embodiments, the stuffer sequence comprises all or a portion of a gene. For example, the stuffer sequence comprises a portion of the human AAT sequence. One skilled in the art would recognize that different portions of a gene (*e.g.*, the human AAT sequence) can be used as a stuffer fragment. For example, the stuffer fragment may be from the 5' end of the gene, the 3' end of the gene, the middle of a gene, a non-coding portion of the gene (*e.g.*, an intron), a coding region of the gene (*e.g.* an exon), or a mixture of non-coding and coding portions of a gene. One skilled in the art would also recognize that all or a portion of stuffer sequence may be used as a stuffer sequence. In some embodiments, the vector comprises a 5' stuffer sequence comprising the nucleotide sequence of SEQ ID NO:18 or a nucleotide sequence with greater than about 80%, 85%, 90%, 95%, or 99% identity to the nucleotide sequence of SEQ ID NO:18. In some embodiments, the vector comprises a 3' stuffer sequence comprising the nucleotide sequence of SEQ ID NO:19 or a nucleotide sequence with greater than about 80%, 85%, 90%, 95%, or 99% identity to the nucleotide sequence of SEQ ID NO:19. In some embodiments, the vector comprises a 5' stuffer sequence comprising the nucleotide sequence of SEQ ID NO:18 or a nucleotide sequence with greater than about 80%, 85%, 90%, 95%, or 99% identity to the nucleotide sequence of SEQ ID NO:18 and comprises a 3' stuffer sequence comprising the nucleotide sequence of SEQ ID NO:19 or a nucleotide sequence with greater than about 80%, 85%, 90%, 95%, or 99% identity to the nucleotide sequence of SEQ ID NO:19.

[0141] In further embodiments, the rAAV particle comprises capsid proteins of AAV1, AAV2, AAV3, AAV4, AAV5, AA6, AAV7, AAV8, AAV9, AAVrh.8, AAVrh8R, AAVrh.10, AAV11, AAV12, AAVrh74, AAVrh74 N502I, AAVrh74 W505R or mutants of these capsid proteins. In some embodiments, a mutant capsid protein maintains the ability to form an AAV capsid. In some embodiments, the rAAV particle comprises AAV5 tyrosine mutant capsid (Zhong L. *et al.*, (2008) *Proc Natl Acad Sci U S A* 105(22):7827-7832. In further embodiments,

the rAAV particle comprises capsid proteins of an AAV serotype from Clades A-F (Gao, *et al.*, J. Virol. 2004, 78(12):6381).

[0142] Different AAV serotypes are used to optimize transduction of particular target cells or to target specific cell types within a particular target tissue (*e.g.*, a diseased tissue). A rAAV particle can comprise viral proteins and viral nucleic acids of the same serotype or a mixed serotype. For example, in some embodiments a rAAV particle can comprise AAV1 capsid proteins and at least one AAV2 ITR or it can comprise AAV2 capsid proteins and at least one AAV1 ITR. Any combination of AAV serotypes for production of a rAAV particle is provided herein as if each combination had been expressly stated herein. In some embodiments, the invention provides rAAV particles comprising an AAV1 capsid and a rAAV vector of the present disclosure (*e.g.*, an expression cassette comprising nucleic acid encoding an RNAi of the present disclosure), flanked by at least one AAV2 ITR. In some embodiments, the invention provides rAAV particles comprising an AAV2 capsid.

[0143] In some aspects, the invention provides viral particles comprising a recombinant self-complementing genome. rAAV particles with self-complementing genomes and methods of use of self-complementing AAV genomes are described in US Patent Nos. 6,596,535; 7,125,717; 7,465,583; 7,785,888; 7,790,154; 7,846,729; 8,093,054; and 8,361,457; and Wang Z., *et al.*, (2003) *Gene Ther* 10:2105-2111, each of which are incorporated herein by reference in its entirety. A rAAV comprising a self-complementing genome will quickly form a double stranded DNA molecule by virtue of its partially complementing sequences (*e.g.*, complementing coding and non-coding strands of a transgene). In some embodiments, the invention provides a rAAV particle comprising an AAV genome, wherein the rAAV genome comprises a first heterologous polynucleotide sequence (*e.g.*, an RNAi of the present disclosure) and a second heterologous polynucleotide sequence (*e.g.*, antisense strand of an RNAi of the present disclosure) wherein the first heterologous polynucleotide sequence can form intrastrand base pairs with the second polynucleotide sequence along most or all of its length. In some embodiments, the first heterologous polynucleotide sequence and a second heterologous polynucleotide sequence are linked by a sequence that facilitates intrastrand basepairing; *e.g.*, a hairpin DNA structure. Hairpin structures are known in the art, for example in miRNA or siRNA molecules. In some embodiments, the first heterologous polynucleotide sequence and a second heterologous polynucleotide sequence are linked by a mutated ITR (*e.g.*, the right ITR). In some embodiments, the ITR comprises the polynucleotide sequence 5'-
CCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCC
GACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGA

-3 (SEQ ID NO:27). The mutated ITR comprises a deletion of the D region comprising the terminal resolution sequence. As a result, on replicating an AAV viral genome, the rep proteins will not cleave the viral genome at the mutated ITR and as such, a recombinant viral genome comprising the following in 5' to 3' order will be packaged in a viral capsid: an AAV ITR, the first heterologous polynucleotide sequence including regulatory sequences, the mutated AAV ITR, the second heterologous polynucleotide in reverse orientation to the first heterologous polynucleotide and a third AAV ITR. In some embodiments, the invention provides AAV viral particles comprising a recombinant viral genome comprising a functional AAV2 ITR, a first polynucleotide sequence encoding an RNAi of the present disclosure, a mutated AAV2 ITR comprising a deletion of the D region and lacking a functional terminal resolution sequence, a second polynucleotide sequence comprising the complementary sequence to the sequence encoding an RNAi of the present disclosure, of the first polynucleotide sequence and a functional AAV2 ITR.

[0144] rAAV particles can be produced using methods known in the art. See, *e.g.*, U.S. Pat. Nos. 6,566,118; 6,989,264; and 6,995,006. In practicing the invention, host cells for producing rAAV particles include mammalian cells, insect cells, plant cells, microorganisms and yeast. Host cells can also be packaging cells in which the AAV rep and cap genes are stably maintained in the host cell or producer cells in which the AAV vector genome is stably maintained. Exemplary packaging and producer cells are derived from 293, A549 or HeLa cells. AAV vectors are purified and formulated using standard techniques known in the art.

[0145] Methods known in the art for production of rAAV vectors include but are not limited to transfection, stable cell line production, and infectious hybrid virus production systems which include adenovirus-AAV hybrids, herpesvirus-AAV hybrids (Conway, JE *et al.*, (1997) *J. Virology* 71(11):8780-8789) and baculovirus-AAV hybrids. rAAV production cultures for the production of rAAV virus particles all require; 1) suitable host cells, including, for example, human-derived cell lines such as HeLa, A549, or 293 cells, or insect-derived cell lines such as SF-9, in the case of baculovirus production systems; 2) suitable helper virus function, provided by wild-type or mutant adenovirus (such as temperature sensitive adenovirus), herpes virus, baculovirus, or a plasmid construct providing helper functions; 3) AAV rep and cap genes and gene products; 4) a nucleic acid (such as a therapeutic nucleic acid) flanked by at least one AAV ITR sequences ; and 5) suitable media and media components to support rAAV production. In some embodiments, the AAV rep and cap gene products may be from any AAV serotype. In general, but not obligatory, the AAV rep gene product is of the same serotype as the ITRs of the rAAV vector genome as long as the rep gene products may function to replicated and package

the rAAV genome. Suitable media known in the art may be used for the production of rAAV vectors. These media include, without limitation, media produced by Hyclone Laboratories and JRH including Modified Eagle Medium (MEM), Dulbecco's Modified Eagle Medium (DMEM), custom formulations such as those described in U.S. Patent No. 6,566,118, and Sf-900 II SFM media as described in U.S. Patent No. 6,723,551, each of which is incorporated herein by reference in its entirety, particularly with respect to custom media formulations for use in production of recombinant AAV vectors. In some embodiments, the AAV helper functions are provided by adenovirus or HSV. In some embodiments, the AAV helper functions are provided by baculovirus and the host cell is an insect cell (*e.g.*, *Spodoptera frugiperda* (Sf9) cells).

[0146] In some embodiments, rAAV particles may be produced by a triple transfection method, such as the exemplary triple transfection method provided infra. Briefly, a plasmid containing a rep gene and a capsid gene, along with a helper adenoviral plasmid, may be transfected (*e.g.*, using the calcium phosphate method) into a cell line (*e.g.*, HEK-293 cells), and virus may be collected and optionally purified. As such, in some embodiments, the rAAV particle was produced by triple transfection of a nucleic acid encoding the rAAV vector, a nucleic acid encoding AAV rep and cap, and a nucleic acid encoding AAV helper virus functions into a host cell, wherein the transfection of the nucleic acids to the host cells generates a host cell capable of producing rAAV particles.

[0147] In some embodiments, rAAV particles may be produced by a producer cell line method, such as the exemplary producer cell line method provided infra (see also (referenced in Martin *et al.*, (2013) *Human Gene Therapy Methods* 24:253-269). Briefly, a cell line (*e.g.*, a HeLa cell line) may be stably transfected with a plasmid containing a rep gene, a capsid gene, and a promoter-heterologous nucleic acid sequence. Cell lines may be screened to select a lead clone for rAAV production, which may then be expanded to a production bioreactor and infected with an adenovirus (*e.g.*, a wild-type adenovirus) as helper to initiate rAAV production. Virus may subsequently be harvested, adenovirus may be inactivated (*e.g.*, by heat) and/or removed, and the rAAV particles may be purified. As such, in some embodiments, the rAAV particle was produced by a producer cell line comprising one or more of nucleic acid encoding the rAAV vector, a nucleic acid encoding AAV rep and cap, and a nucleic acid encoding AAV helper virus functions.

[0148] In some aspects, a method is provided for producing any rAAV particle as disclosed herein comprising (a) culturing a host cell under a condition that rAAV particles are produced, wherein the host cell comprises (i) one or more AAV package genes, wherein each said AAV

packaging gene encodes an AAV replication and/or encapsidation protein; (ii) an rAAV pro-vector comprising a nucleic acid encoding an RNAi of the present disclosure as described herein flanked by at least one AAV ITR, and (iii) an AAV helper function; and (b) recovering the rAAV particles produced by the host cell. In some embodiments, the RNAi comprises the nucleotide sequence of SEQ ID NO:7. In some embodiments, said at least one AAV ITR is selected from the group consisting of AAV ITRs are AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAVrh74, AAVrh74 N502I, AAVrh74 W505R, AAV2R471A, AAV DJ, a goat AAV, bovine AAV, or mouse AAV capsid serotype ITRs or the like. In some embodiments, said encapsidation protein is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6 (*e.g.,* a wild-type AAV6 capsid, or a variant AAV6 capsid such as ShH10, as described in U.S. PG Pub. 2012/0164106), AAV7, AAV8, AAVrh8, AAVrh8R, AAV9 (*e.g.,* a wild-type AAV9 capsid, or a modified AAV9 capsid as described in U.S. PG Pub. 2013/0323226), AAV10, AAVrh10, AAV11, AAV12, AAVrh74 (*e.g.,* a wild-type AAVrh74 capsid, or a variant AAVrh74 capsid, such as AAVrh74 N502I or AAVrh74 W505R as described in WO2019178412, incorporated by reference in its entirety), a tyrosine capsid mutant, a heparin binding capsid mutant, an AAV2R471A capsid, an AAVAAV2/2-7m8 capsid, an AAV DJ capsid (*e.g.,* an AAV-DJ/8 capsid, an AAV-DJ/9 capsid, or any other of the capsids described in U.S. PG Pub. 2012/0066783), AAV2 N587A capsid, AAV2 E548A capsid, AAV2 N708A capsid, AAV V708K capsid, goat AAV capsid, AAV1/AAV2 chimeric capsid, bovine AAV capsid, mouse AAV capsid, rAAV2/HBoV1 capsid, or an AAV capsid described in U.S. Pat. No. 8,283,151 or International Publication No. WO/2003/042397. In some embodiments, a mutant capsid protein maintains the ability to form an AAV capsid. In some embodiments, the encapsidation protein is an AAV5 tyrosine mutant capsid protein. In further embodiments, the rAAV particle comprises capsid proteins of an AAV serotype from Clades A-F. In some embodiments, the rAAV particles comprise an AAVrh74 N502I capsid and a recombinant genome comprising AAV2 ITRs and nucleic acid encoding an RNAi of the present disclosure. In some embodiments, the rAAV particles comprise an AAVrh74 W505R capsid and a recombinant genome comprising AAV2 ITRs and nucleic acid encoding an RNAi of the present disclosure. In a further embodiment, the rAAV particles are purified. The term "purified" as used herein includes a preparation of rAAV particles devoid of at least some of the other components that may also be present where the rAAV particles naturally occur or are initially prepared from. Thus, for example, isolated rAAV particles may be prepared using a purification technique to enrich it from a source mixture, such as a culture lysate or production culture supernatant. Enrichment can be measured in a variety of

ways, such as, for example, by the proportion of DNase-resistant particles (DRPs) or genome copies (gc) present in a solution, or by infectivity, or it can be measured in relation to a second, potentially interfering substance present in the source mixture, such as contaminants, including production culture contaminants or in-process contaminants, including helper virus, media components, and the like.

[0149] Also provided herein are pharmaceutical compositions comprising a rAAV particle comprising a transgene encoding an RNAi of the present disclosure and a pharmaceutically acceptable carrier. The pharmaceutical compositions may be suitable for any mode of administration described herein. A pharmaceutical composition of a rAAV particle comprising a nucleic acid encoding an RNAi of the present disclosure can be introduced systemically. For example, a recombinant viral particle comprising a nucleic acid encoding an RNAi of the present disclosure can be administered intravenously, intra-arterially, subcutaneously or interperitoneally.

[0150] In some embodiments, the pharmaceutical compositions comprising a recombinant viral particle comprising a transgene encoding an RNAi of the present disclosure described herein and a pharmaceutically acceptable carrier is suitable for administration to human. Such carriers are well known in the art (see, *e.g.*, Remington's Pharmaceutical Sciences, 15th Edition, pp. 1035-1038 and 1570-1580). In some embodiments, the pharmaceutical compositions comprising a rAAV described herein and a pharmaceutically acceptable carrier is suitable for systemic injection into a mammal.

[0151] Such pharmaceutically acceptable carriers can be sterile liquids, such as water and oil, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, and the like. Saline solutions and aqueous dextrose, polyethylene glycol (PEG) and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. The pharmaceutical composition may further comprise additional ingredients, for example preservatives, buffers, tonicity agents, antioxidants and stabilizers, nonionic wetting or clarifying agents, viscosity-increasing agents, and the like. The pharmaceutical compositions described herein can be packaged in single unit dosages or in multidosage forms. The compositions are generally formulated as sterile and substantially isotonic solution.

Articles of Manufacture and Kits

[0152] Also provided are kits or articles of manufacture for use in the methods described herein. In aspects, the kits comprise the compositions described herein (*e.g.*, a rAAV particle of the present disclosure comprising nucleic acid encoding an RNAi of the present disclosure) in

suitable packaging. Suitable packaging for compositions described herein are known in the art, and include, for example, vials (such as sealed vials), vessels, ampules, bottles, jars, flexible packaging (*e.g.*, sealed Mylar or plastic bags), and the like. These articles of manufacture may further be sterilized and/or sealed.

[0153] The present invention also provides kits comprising compositions described herein and may further comprise instruction(s) on methods of using the composition, such as uses described herein. The kits described herein may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for performing any methods described herein. For example, in some embodiments, the kit comprises a composition of recombinant viral particles comprising a transgene encoding an RNAi of the present disclosure for delivery of an effective amount of the rAAV particles to a mammal, a pharmaceutically acceptable carrier suitable for injection into the mammal, and one or more of: a buffer, a diluent, a filter, a needle, a syringe, and a package insert with instructions for performing injections into the mammal. In some embodiments, the kit comprising instructions for treating DM-1 with the rAAV particles described herein. In some embodiments, the kit comprising instructions for using the rAAV particles described herein according to any one of the methods described herein.

EXEMPLARY EMBODIMENTS

[0154] The invention includes the following enumerated exemplary embodiments.

[0155] 1. An RNAi comprising a first strand and a second strand, wherein a) the first strand and the second strand form a duplex; b) the first strand comprises a guide region, wherein the guide region comprises nucleic acid with the sequence 5'- AGUCGAAGACAGUUCUAGGGU-3' (SEQ ID NO:1) or with a sequence with about 90% identity to the sequence of SEQ ID NO:1; and c) the second strand comprises a non-guide region.

[0156] 2. The RNAi of embodiment 1, wherein the non-guide region comprises nucleic acid with the sequence 5' ACCCUAGAUGUCUUCGAUU-3' (SEQ ID NO:2) or a with a sequence with about 90% identity to the sequence of SEQ ID NO:2.

[0157] 3. The RNAi of embodiment 1 or 2, wherein the first strand comprises nucleic acid with the sequence of SEQ ID NO:1 and the non-guide region comprises nucleic acid with the sequence of SEQ ID NO:2.

[0158] 4. The RNAi of any one of embodiments 1-3, wherein the first strand and the second strand are linked by means of a RNA linker capable of forming a loop structure.

[0159] 5. The RNAi of embodiment 4, wherein the RNA linker comprises from about 4 to about 50 nucleotides.

[0160] 6. The RNAi of embodiment 4 or 5, wherein the loop structure comprises from about 4 to about 20 nucleotides.

[0161] 7. The RNAi of any one of embodiments 4-6, wherein the loop structure comprises nucleic sequence with of SEQ ID NO:3 or with a sequence with about 90% identity to the sequence of SEQ ID NO:3.

[0162] 8. The RNAi of any one of embodiments 4-7, wherein the RNAi comprises 5' to 3' the second strand, the RNA linker, and the first strand.

[0163] 9. The RNAi of any one of embodiments 4-7, wherein the RNAi comprises 5' to 3' the first strand, the RNA linker, and the second strand.

[0164] 10. The RNAi of any one of embodiments 1-8, wherein the RNAi comprises nucleic acid with the sequence of SEQ ID NO:7 or with a sequence with about 90% identity to the sequence of SEQ ID NO:7.

[0165] 11. The RNAi of any one of embodiments 1-10, wherein the RNAi is a small inhibitory RNA (siRNA), a microRNA (miRNA), or a small hairpin RNA (shRNA).

[0166] 12. The RNAi of any one of embodiment 1-11, wherein the RNAi further comprises a scaffold.

[0167] 13. The RNAi of embodiment 12, wherein the scaffold comprises all or a portion of the nucleic acid of SEQ ID No: 11.

[0168] 14. The RNAi of embodiment 13, wherein the miRNA is embedded within the scaffold.

[0169] 15. The RNAi of embodiments 14, wherein the scaffold has a 5'arm, wherein the 5' arm is located 5' to the nucleic acid encoding the RNAi, and a 3'arm, wherein the 3' arm is located 3' to the nucleic acid encoding the RNAi.

[0170] 16. The RNAi of any one of embodiments 12-15, wherein the scaffold is a miR-155 scaffold.

[0171] 17. The RNAi of any one of embodiments 12-16, wherein the miR-155 scaffold comprises the nucleic acid of SEQ ID NO:9 or a sequence with about 90% identity to the sequence of SEQ ID NO:9 located 5' to the RNAi.

[0172] 18. The RNAi of any one of embodiments 12-17, wherein the miR-155 scaffold comprises the nucleic acid of SEQ ID NO:10 or a sequence with about 90% identity to the sequence of SEQ ID NO:10 located 3' to the RNAi.

[0173] 19. The RNAi of any one of embodiments 1-18, wherein the RNAi targets RNA encoding a polypeptide associated with myotonic dystrophy-1 (DM1).

[0174] 20. The RNAi of embodiment 19, wherein the polypeptide is dystrophia myotonica protein kinase (DMPK).

[0175] 21. The RNAi of embodiment 20, wherein the DMPK comprises a mutation associated with DM 1.

[0176] 22. The RNAi of embodiment 20 or 21, wherein the gene encoding DMPK comprises five or more CTG trinucleotide repeats.

[0177] 23. An expression cassette comprising nucleic acid encoding the RNAi of any one of embodiments 1-22.

[0178] 24. The expression cassette of embodiment 23, wherein the nucleic acid encoding the RNAi is operably linked to a promoter.

[0179] 25. The expression cassette of embodiment 24, wherein the promoter is a muscle-specific promotor.

[0180] 26. The expression cassette of embodiment 24 or 25, wherein the promoter is a desmin promoter or variant thereof.

[0181] 27. The expression cassette of embodiment 26, wherein the desmin promoter comprises one or more enhancer elements and the promoter for the human desmin gene.

[0182] 28. The expression cassette of embodiment 26 or 27, wherein the desmin promoter comprises two enhancer elements and the promoter for the human desmin gene.

[0183] 29. The expression cassette of any one of embodiments 26-28, wherein the desmin promoter comprises one or more Byrne enhancer elements and/or one or more Paulin enhancer elements.

[0184] 30. The expression cassette of any one of embodiments 26-29, wherein the desmin promoter comprises one or more enhancer elements comprising the nucleotide sequence of SEQ ID NO:21 or a nucleotide sequence with about 90% identity to the sequence of SEQ ID NO:21 and/or one or more enhancer elements comprising the nucleotide sequence of SEQ ID NO:22 or a nucleotide sequence with about 90% identity to the sequence of SEQ ID NO:22.

[0185] 31. The expression cassette of any one of embodiments 26-30, wherein the desmin promoter comprises the nucleotide sequence of SEQ ID NO:12 or a sequence with about 90% identity to the nucleotide sequence of SEQ ID NO:12.

[0186] 32. The expression cassette of any one of embodiments 23-31, wherein the expression cassette further comprises an intron.

[0187] 33. The expression cassette of embodiment 32, wherein the intron is a rabbit β-globin intron.

[0188] 34. The expression cassette of embodiment 32 or 33, wherein the intron comprises the nucleotide sequence of SEQ ID NO:13 or a sequence with about 90% identity to the sequence of SEQ ID NO:13.

[0189] 35. The expression cassette of any one of embodiments 32-34, wherein the nucleic acid encoding the RNAi is embedded in the intron.

[0190] 36. The expression cassette of embodiment 35, wherein the intron comprises a 5' arm and a 3' arm, wherein the 5' arm is located 5' to the nucleic acid encoding the RNAi and the 3' arm is located 3' to the nucleic acid encoding the RNAi.

[0191] 37. The expression cassette of embodiment 36, wherein the 5' arm of the intron comprises the nucleotide sequence of SEQ ID NO:14 or a sequence with about 90% identity to the sequence of SEQ ID NO:14.

[0192] 38. The expression cassette of embodiment 36 or 37, wherein the 3' arm of the intron comprises the nucleotide sequence of SEQ ID NO:15 or a sequence with about 90% identity to the sequence of SEQ ID NO:15.

[0193] 39. The expression cassette of any one of embodiments 23-38, wherein the expression cassette further comprises a polyadenylation signal.

[0194] 40. The expression cassette of embodiment 39 wherein the polyadenylation signal is a bovine growth hormone polyadenylation signal, an SV40 polyadenylation signal, or a HSV TK pA.

[0195] 41. The expression cassette of embodiment 40, wherein the polyadenylation signal is a minimal bovine growth hormone polyadenylation signal.

[0196] 42. The expression cassette of any one of embodiments 39-41, wherein the bovine growth hormone polyadenylation signal comprises the nucleotide sequence of SEQ ID NO:16 or a sequence with about 90% identity to the sequence of SEQ ID NO:16.

[0197] 43. The expression cassette of any one of embodiments 23-42, wherein the expression cassette comprises the nucleotide sequence of SEQ ID NO:17 or a sequence with about 90% identity to the sequence of SEQ ID NO:17.

[0198] 44. An expression cassette, wherein the expression cassette comprises a modified desmin promoter, wherein the modified desmin promoter comprises one or more enhancer elements and the promoter for the human desmin gene.

[0199] 45. The expression cassette of embodiment 44, wherein the modified desmin promoter comprises two enhancer elements and the promoter for the human desmin gene.

[0200] 46. The expression cassette of embodiment 44 or 45, wherein the modified desmin promoter comprises one or more Byrne enhancer elements and/or one or more Paulin enhancer elements.

[0201] 47. The expression cassette of any one of embodiments 44-46, wherein the modified desmin promoter comprises one or more enhancer elements comprising the nucleotide sequence of SEQ ID NO:21 or a nucleotide sequence with about 90% identity to the sequence of SEQ ID NO:21 and/or one or more enhancer elements comprising the nucleotide sequence of SEQ ID NO:22 or a nucleotide sequence with about 90% identity to the sequence of SEQ ID NO:22.

[0202] 48. The expression cassette of any one of embodiments 44-47, wherein the desmin promoter comprises the nucleotide sequence of SEQ ID NO:12 or a sequence with about 90% identity to the nucleotide sequence of SEQ ID NO:12.

[0203] 49. The expression cassette of any one of embodiments 44-48, wherein the expression cassette further comprises an intron.

[0204] 50. The expression cassette of embodiment 49, wherein the intron is a rabbit β-globin intron.

[0205] 51. The expression cassette of embodiment 49 or 50, wherein the intron comprises the nucleotide sequence of SEQ ID NO:13 or a sequence with about 90% identity to the sequence of SEQ ID NO:13.

[0206] 52. The expression cassette of any one of embodiments 44-51, wherein the nucleic acid encoding the transgene is embedded in the intron.

[0207] 53. The expression cassette of embodiment 52, wherein the intron comprises a 5' arm and a 3' arm, wherein the 5' arm is located 5' to the nucleic acid encoding the transgene and the 3' arm is located 3' to the nucleic acid encoding the transgene.

[0208] 54. The expression cassette of embodiment 53, wherein the 5' arm of the intron comprises the nucleotide sequence of SEQ ID NO:14 or a sequence with about 90% identity to the sequence of SEQ ID NO:14.

[0209] 55. The expression cassette of embodiment 53 or 54, wherein the 3' arm of the intron comprises the nucleotide sequence of SEQ ID NO:15 or a sequence with about 90% identity to the sequence of SEQ ID NO:15.

[0210] 56. The expression cassette of any one of embodiments 44-55, wherein the expression cassette further comprises a polyadenylation signal.

[0211] 57. The expression cassette of embodiment 56, wherein the polyadenylation signal is a bovine growth hormone polyadenylation signal, an SV40 polyadenylation signal, or a HSV TK pA.

[0212] 58. The expression cassette of embodiment 57, wherein the polyadenylation signal is a minimal bovine growth hormone polyadenylation signal.

[0213] 59. The expression cassette of any one of embodiments 56-58, wherein the bovine growth hormone polyadenylation signal comprises the nucleotide sequence of SEQ ID NO:16 or a sequence with about 90% identity to the sequence of SEQ ID NO:16.

[0214] 60. The expression cassette of any one of embodiments 44-59, wherein the transgene encodes a polypeptide or a nucleic acid.

[0215] 61. The expression cassette of any one of embodiments 44-60, wherein the transgene encodes an RNAi.

[0216] 62. A vector comprising the expression cassette of any one of embodiments 23-61.

[0217] 63. The vector of embodiment 62, wherein the expression cassette is flanked by one or more stuffer nucleic acid sequences.

[0218] 64. The vector of embodiment 63, wherein the one or more stuffer nucleic acid sequences is derived from the human SerpinA1 gene.

[0219] 65. The vector of embodiment 63 or 64, wherein a stuffer nucleic acid sequence located 5' to the expression cassette is derived from the human SerpinA1 gene.

[0220] 66. The vector of any one of embodiments 63-65, wherein a stuffer sequence located 5' to the expression cassette comprises the nucleotide sequence of SEQ ID NO:18 or a sequence with about 90% identity to the sequence of SEQ ID NO:18.

[0221] 67. The vector of any one of embodiments 63-66, wherein a stuffer nucleic acid sequence located 3' to the expression cassette is derived from the human SerpinA1 gene.

[0222] 68. The vector of any one of embodiments 63-67, wherein a stuffer sequence located 3' to the expression cassette comprises the nucleotide sequence of SEQ ID NO:19 or a sequence with about 90% identity to the sequence of SEQ ID NO:19.

[0223] 69. The vector of any one of embodiments 62-68, wherein the vector is a recombinant adeno-associated virus (rAAV) vector.

[0224] 70. The rAAV vector of embodiment 69, wherein the expression cassette is flanked by one or more AAV inverted terminal repeat (ITR) sequences.

[0225] 71. The rAAV vector of embodiment 70, wherein the expression cassette is flanked by two AAV ITRs.

[0226] 72. The rAAV vector of embodiment 70 or 71, wherein the AAV ITRs are AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV2R471A, AAV DJ, a goat AAV, bovine AAV, or mouse AAV serotype ITRs.

[0227] 73. The rAAV vector of any one of embodiments 70-72, wherein the AAV ITRs are AAV2 ITRs.

[0228] 74. The rAAV vector of any one of embodiments 69-73, wherein the rAAV vector comprises the nucleotide sequence of SEQ ID NO:20 or a sequence with about 90% identity to the sequence of SEQ ID NO:20.

[0229] 75. The rAAV vector of any one of embodiments 69-74, wherein the vector is a self-complementary rAAV vector.

[0230] 76. A cell comprising the expression cassette of any one of embodiments 23-61, the vector of any one of embodiments 62-68, or the rAAV vector of any one of embodiments 69-75.

[0231] 77. A viral particle comprising the vector of any one of embodiments 62-68.

[0232] 78. A recombinant AAV particle comprising the rAAV vector of any one of embodiments 69-75.

[0233] 79. The rAAV particle of embodiment 78, wherein the AAV viral particle comprises an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAVrh74, AAVrh74 N502I, AAVrh74 W505R, AAV2R471A, AAV2/2-7m8, AAV DJ, AAV2 N587A, AAV2 E548A, AAV2 N708A, AAV

V708K, AAV2-HBKO, AAVDJ8, AAVPHP.B, AAVPHP.eB, AAVBR1, AAVHSC15, AAVHSC17, a goat AAV, AAV1/AAV2 chimeric, bovine AAV, or mouse AAV capsid rAAV2/HBoV1 serotype capsid.

[0234] 80. The rAAV particle of embodiment 78 or 79, wherein the ITR and the capsid of the rAAV viral particle are derived from the same AAV serotype.

[0235] 81. The rAAV particle of embodiment 78 or 79, wherein the ITR and the capsid of the rAAV viral particle are derived from different AAV serotypes.

[0236] 82. The rAAV particle of embodiment 78, 79 or 81, wherein the AAV viral particle comprises a AAVrh74 N502I serotype capsid.

[0237] 83. The rAAV particle of embodiment 82, wherein the ITR is an AAV2 ITR and the capsid of the rAAV particle is an AAVrh74 N502I serotype capsid.

[0238] 84. The rAAV particle of embodiment 78, 79, or 81, wherein the AAV viral particle comprises a AAVrh74 W505R serotype capsid.

[0239] 85. The rAAV particle of embodiment 84, wherein the ITR is an AAV2 ITR and the capsid of the rAAV particle is an AAVrh74 W505R serotype capsid.

[0240] 86. An rAAV particle comprising an rAAV vector and a capsid, wherein the rAAV vector comprises the following nucleic acids 5' to 3', an AAV2 ITR, nucleic acid encoding a stuffer nucleic acid sequence from the human serpinA1 gene, a Byrne desmin enhancer element, a Paulin desmin enhancer element, a desmin promoter, a 5' arm of a rabbit β-globin intron, a 5' miR155 scaffold sequence, a $DMPK^{204}$ miRNA guide sequence, a miR155 terminal loop sequence, a $DMPK^{204}$ miRNA passenger sequence, a 3' miR155 scaffold sequence, a 3' arm of a rabbit β-globin intron, a minimal bovine growth hormone polyadenylation sequence, nucleic acid encoding a stuffer nucleic acid sequence from the human serpinA1 gene, and an AAV2 ITR; and wherein the capsid is an AAVrh74 N502I capsid.87.An rAAV particle comprising an rAAV vector, wherein the rAAV vector comprises the following nucleic acids 5' to 3', an AAV2 ITR comprising the polynucleotide sequence of SEQ ID NO:43, nucleic acid encoding a stuffer nucleic acid sequence from the human serpinA1 gene comprising the polynucleotide sequence of SEQ ID NO:18, a Byrne desmin enhancer element comprising the polynucleotide sequence of SEQ ID NO:21, a Paulin desmin enhancer element comprising the polynucleotide sequence of SEQ ID NO:22, a desmin promoter comprising the polynucleotide sequence of SEQ ID NO:23, a 5' arm of a rabbit β-globin intron comprising the polynucleotide sequence of SEQ ID NO:14, a 5' miR155 scaffold sequence comprising the polynucleotide sequence of SEQ ID NO:40, a

DMPK^{204} miRNA guide sequence comprising the polynucleotide sequence of SEQ ID NO:4, a miR155 terminal loop sequence comprising the polynucleotide sequence of SEQ ID NO:6, a DMPK^{204} miRNA passenger sequence comprising the polynucleotide sequence of SEQ ID NO:5, a 3' miR155 scaffold sequence comprising the polynucleotide sequence of SEQ ID NO:41, a 3' arm of a rabbit β-globin intron comprising the polynucleotide sequence of SEQ ID NO:15, a minimal bovine growth hormone polyadenylation sequence comprising the polynucleotide sequence of SEQ ID NO:16, nucleic acid encoding a stuffer nucleic acid sequence from the human serpinA1 gene comprising the polynucleotide sequence of SEQ ID NO:19, and an AAV2 ITR comprising the polynucleotide sequence of SEQ ID NO:49; and wherein the capsid is an AAVrh74 N502I capsid.

[0241] 88. The rAAV particle of embodiment 86 or 87, wherein the AAVrh74 N502I capsid comprises capsid proteins comprising the amino acid sequence of SEQ ID NO:50.

[0242] 89. An rAAV particle comprising an rAAV vector and a capsid, wherein the rAAV vector comprises the following nucleic acids 5' to 3', an AAV2 ITR, nucleic acid encoding a stuffer nucleic acid sequence from the human serpinA1 gene, a Byrne desmin enhancer element, a Paulin desmin enhancer element, a desmin promoter, a 5' arm of a rabbit β-globin intron, a 5' miR155 scaffold sequence, a DMPK^{204} miRNA guide sequence, a miR155 terminal loop sequence, a DMPK^{204} miRNA passenger sequence, a 3' miR155 scaffold sequence, a 3' arm of a rabbit β-globin intron, a minimal bovine growth hormone polyadenylation sequence, nucleic acid encoding a stuffer nucleic acid sequence from the human serpinA1 gene, and an AAV2 ITR; and wherein the capsid is an AAVrh74 W505R capsid.

[0243] 90. An rAAV particle comprising an rAAV vector, wherein the rAAV vector comprises the following nucleic acids 5' to 3', an AAV2 ITR comprising the polynucleotide sequence of SEQ ID NO:43, nucleic acid encoding a stuffer nucleic acid sequence from the human serpinA1 gene comprising the polynucleotide sequence of SEQ ID NO:18, a Byrne desmin enhancer element comprising the polynucleotide sequence of SEQ ID NO:21, a Paulin desmin enhancer element comprising the polynucleotide sequence of SEQ ID NO:22, a desmin promoter comprising the polynucleotide sequence of SEQ ID NO:23, a 5' arm of a rabbit β-globin intron comprising the polynucleotide sequence of SEQ ID NO:14, a 5' miR155 scaffold sequence comprising the polynucleotide sequence of SEQ ID NO:40, a DMPK^{204} miRNA guide sequence comprising the polynucleotide sequence of SEQ ID NO:4, a miR155 terminal loop sequence comprising the polynucleotide sequence of SEQ ID NO:6, a DMPK^{204} miRNA passenger sequence comprising the polynucleotide sequence of SEQ ID NO:5, a 3' miR155

scaffold sequence comprising the polynucleotide sequence of SEQ ID NO:41, a 3' arm of a rabbit β-globin intron comprising the polynucleotide sequence of SEQ ID NO:15, a minimal bovine growth hormone polyadenylation sequence comprising the polynucleotide sequence of SEQ ID NO:16, nucleic acid encoding a stuffer nucleic acid sequence from the human serpinA1 gene comprising the polynucleotide sequence of SEQ ID NO:19, and an AAV2 ITR comprising the polynucleotide sequence of SEQ ID NO:49; and wherein the capsid is an AAVrh74 W505R capsid.

[0244] 91. The rAAV particle of embodiment 89 or 90, wherein the AAVrh74 W505R capsid comprises capsid proteins comprising the amino acid sequence of SEQ ID NO:52.

[0245] 92. A composition comprising the viral particle embodiment 77 or the rAAV particle of any one of embodiments 78-91.

[0246] 93. A pharmaceutical composition comprising the viral particle embodiment 77 or the rAAV particle of any one of embodiments 78-91.

[0247] 94. The composition of embodiment 92 or 93, wherein the composition further comprises a pharmaceutically acceptable carrier.

[0248] 95. A modified desmin promoter, wherein the modified desmin promoter comprises one or more enhancer elements and the promoter for the human desmin gene.

[0249] 96. The modified desmin promoter of embodiment 95, wherein the modified desmin promoter comprises two enhancer elements and the promoter for the human desmin gene.

[0250] 97. The modified desmin promoter of embodiment 95 or 96, wherein the modified desmin promoter comprises one or more Byrne enhancer elements and/or one or more Paulin enhancer elements.

[0251] 98. The modified desmin promoter of any one of embodiments 95-97, wherein the modified desmin promoter comprises one or more enhancer element comprising the nucleotide sequence of SEQ ID NO:21 or a sequence with about 90% identity to the sequence of SEQ ID NO:21 and/or one or more enhancer element comprising the nucleotide sequence of SEQ ID NO:22 or a sequence with about 90% identity to the sequence of SEQ ID NO:22.

[0252] 99. The modified desmin promoter of any one of embodiments 95-98, wherein the modified desmin promoter comprises the nucleotide sequence of SEQ ID NO:12 or a sequence with about 90% identity to the sequence of SEQ ID NO:12.

[0253] 100. A kit comprising the RNAi of any one of embodiments 1-22.

[0254] 101. A kit comprising the viral particle of embodiment 77 or the AAV particle of any one of embodiments 78-91.

[0255] 102. A kit comprising the composition of any one of embodiments 92-94.

[0256] 103. The kit of any one of embodiments 100-102, further comprising instructions for use.

[0257] 104. A method for treating myotonic dystrophy-1 (DM1) in a mammal in need thereof comprising administering to the mammal an effective amount of the RNAi of any one of embodiments 1-22.

[0258] 105. A method for inhibiting the expression of dystrophia myotonica protein kinase (DMPK) in a mammal with DM-1 in need thereof comprising administering to the mammal an effective amount of a the RNAi of any one of embodiments 1-22.

[0259] 106. A method for inhibiting the accumulation of DMPK RNA in a cell of a mammal with DM-1 in need thereof comprising administering to the mammal an effective amount of a the RNAi of any one of embodiments 1-22.

[0260] 107. A method for treating myotonic dystrophy-1 (DM1) in a mammal in need thereof comprising administering to the mammal an effective amount of a the viral particle of embodiment 77 or the rAAV particle of any one of embodiments 78-91.

[0261] 108. A method for inhibiting the expression of dystrophia myotonica protein kinase (DMPK) in a mammal with DM-1 in need thereof comprising administering to the mammal an effective amount of a the viral particle of embodiment 77 or an effective amount of a the rAAV particle of any one of embodiments 78-91.

[0262] 109. A method for inhibiting the accumulation of DMPK RNA in a cell of a mammal with DM-1 in need thereof comprising administering to the mammal an effective amount of a the viral particle of embodiment 77 or an effective amount of a the rAAV particle of any one of embodiments 78-91.

[0263] 110. The method of any one of embodiments 102-104, wherein the effective amount of the viral particle or rAAV particle is a dose of about 1×10^{8} to about 2×10^{13} genome copies/mL.

[0264] 111. The method of embodiment 110, wherein the dose is about 5×10^{12} genome copies/mL.

[0265] 112. The method of embodiment 110, wherein the dose is about 1 x 10^{13} genome copies/mL.

[0266] 113. The method of embodiment 110, wherein the dose is about 2 x 10^{13} genome copies/mL.

[0267] 114. The method of any one of embodiments 107-109, wherein the effective amount of the viral particle or rAAV particle is a dose of about 1 x 10^{8} to about 2 x 10^{14} genome copies/kg of body weight.

[0268] 115. The method of embodiment 114, wherein the dose is about 5 x 10^{13} genome copies/kg of body weight.

[0269] 116. The method of embodiment 114, wherein the dose is about 1 x 10^{14} genome copies/kg of body weight.

[0270] 117. The method of embodiment 114, wherein the dose is about 2 x 10^{14} genome copies/kg of body weight.

[0271] 118. A method for treating myotonic dystrophy-1 (DM1) in a mammal in need thereof comprising administering to the mammal an effective amount of the composition of any one of embodiments 92-94.

[0272] 119. A method for inhibiting the expression of dystrophia myotonica protein kinase (DMPK) in a mammal with DM-1 in need thereof comprising administering to the mammal an effective amount of the composition of any one of embodiments 92-94.

[0273] 120. A method for inhibiting the accumulation of DMPK RNA in a cell of a mammal with DM-1 in need thereof comprising administering to the mammal an effective amount of the composition of any one of embodiments 92-94.

[0274] 121. The method of any one of embodiments 104-106, wherein the RNAi is administered in combination with an immunosuppressive agent, wherein the immunosuppressive agent is administered before, at the same time, and/or after administration of the RNAi.

[0275] 122. The method of any one of embodiments 107-109, wherein the viral particle or the rAAV particle is administered in combination with an immunosuppressive agent, wherein the immunosuppressive agent is administered before, at the same time, and/or after administration of the viral particle or the rAAV particle.

[0276] 123. The method of any one of embodiments 118-120, wherein the composition is administered in combination with an immunosuppressive agent, wherein the immunosuppressive agent is administered before, at the same time, and/or after administration of the composition.

EXAMPLES

[0277] The presently disclosed subject matter will be better understood by reference to the following Examples, which are provided as exemplary of the invention, and not by way of limitation.

Example 1: Generation of the amiR-DMPK^{204} expression cassettes

[0278] A single-stranded AAV viral vector encoding a microRNA (amiR-DMPK^{204}) designed to target the DMPK gene was generated (**FIG. 1A**). The construct was designed such that the amiR-DMPK^{204} microRNA was embedded in an optimized microRNA backbone, miR155 (BLOCK-iT; Thermofisher Catalog nos. K4935-00, K4936-00, K4937-00, K4938-00), and is hereafter "miR155 amiR-DMPK^{204}" or "amiR155-DMPK^{204}". miR155 amiR-DMPK^{204} is flanked by rabbit β-globin intron sequences and was placed under the regulation of a hybrid muscle promoter (nDes).

[0279] The nDesmin promoter comprising the Byrne desmin enhancer, one copy of the Paulin desmin enhancer (-973 to -693) and the promoter of the human Desmin gene (−228 to +75) were synthesized by conventional oligonucleotide synthesis (Genscript, USA).

[0280] A bovine growth hormone polyadenylation sequence was placed 3' of the intron-flanked amiR-DMPK^{204} microRNA (minBGHpA). A filler sequence ("stuffer") was included. The whole gene cassette is flanked by wild-type AAV serotype 2 Inverted Terminal Repeats (ITRs) sequences, for DNA rescue and replication, as well as packaging into an AAV capsid. The sequences were engineered into the ITR plasmid which was used to generate the vector for *in vivo* efficacy studies.

5' and 3' ITR sequences

[0281] The ITR sequences were the AAV2 wild-type sequence of 145bp. The 3' ITR (downstream of expression cassette) was in the Flip orientation (GenBank: LQ493091.1). The 5' ITR (upstream of the expression cassette) was in the Flop orientation (145bp) (Miller *et al.*, 2004, *Nature Genetics* 36.7 (2004): 767-773). The accuracy of the sequence was confirmed by Sanger sequencing.

nDes Promoter

[0282] The nDes promoter was constructed using desmin promoter elements shown in the literature (Li and Paulin, *et. al.* 1991, *J Biol Chem.* 266.10: 6562-6570). The nDes promoter comprises one copy of the Byrne desmin enhancer, one copy of the Paulin desmin enhancer (-973 to -693) and the promoter of the human desmin gene (−228 to +75).

5' and 3' arms of the rabbit β-globin intron

[0283] This intron was used to flank the amiR-DMPK^{204} cassettes because intronic expression of miRNAs is known to enhance target knockdown.

amiR-DMPK^{204} with the miR155 scaffold (miR155-amiRDMPK^{204})

[0284] Endogenous miRNAs are hairpin-like secondary structures found in many primary RNA transcripts (pri-miRNAs). In the nucleus, the microprocessor, Drosha/DGCR8 complex binds and cleaves the basal stem of pri-miRNAs to liberate the stem-loop precursor miRNA (pre-miRNA). Pre-miRNAs are then exported from the nucleus where the loop is cleaved by Dicer/TRBP to form a mature RNA duplex. The guide strand, also known as the targeting strand, is separated from the passenger strand and loaded onto an argonaute protein in the RNA induced silencing complex (RISC), which then targets complementary mRNA transcripts for degradation or translational repression.

[0285] The amiR-DMPK^{204} sequence was identified as target for DM1 therapy. The target sequence of amiR-DMPK^{204} is located upstream of the "CUG" repeat sequences within the 3' UTR of the DMPK nucleotide sequence. Therefore, amiR-DMPK^{204} can suppress both wild-type and mutant DMPK transcripts.

[0286] Additionally, the amiR-DMPK^{204} target region is conserved in non-human primates (NHPs-cynomolgus monkey), and humans, allowing pre-clinical assessment of DMPK knockdown in NHPs (**FIG. 11**). The amiR-DMPK^{204} microRNA was evaluated and it was shown that the miR155 scaffold has efficient guide processing with minimal passenger strand processing, reducing the likelihood of off-target effects (*see* Example 2 below).

[0287] The final construct that was selected for development was amiR-DMPK^{204} with the miR155 scaffold (amiR155-DMPK^{204}), where the guide strand, when processed, targeted DMPK mRNA for degradation. The engineered pre-miRNA sequence structure is based on the murine miR-155 sequence (Lagos-Quintana *et al.*, 2002, *Current Biology*, 12:9, 735-739). The 5' and 3' flanking regions derived from the miR-155 transcript were inserted in the vector to preserve as much as possible of the miR-155 structure. The stem-loop structure was optimized and a 2

nucleotide internal loop resulted in higher knockdown rate than the 5 nucleotide / 3 nucleotide internal loop found in native miR-155 molecule (source: BLOCK-iT™ Pol II miR RNAi Expression Vector, (Invitrogen)). The vector, nDes-miR155-amiR-DMPK^{204}, in the context of a AAV capsid, was shown to have potent in vivo activity in the DMSXL mouse model of DM1 (*see* Example 3 below).

minBGH polyA

[0288] A minimal BGH polyA site of 186 bps was inserted downstream of the amiR-DMPK^{204} sequence to allow transcription termination and polyadenylation of the mRNA.

Stuffer sequence from A1AT Intron

[0289] A stuffer sequence from the alpha-1 antitrypsin gene intron sequence 4 was used to bring the gene cassette to the packaging limits for rAAV vectors.

Cloning of the ITR plasmid with the nDes-miR155- amiR-DMPK^{204} cassette (nDes-miR155-amiR-DMPK^{204})

[0290] A 1938 bp ITR- ITR sequence of the expression construct, nDes- miR155-amiR-DMPK204-minBGHpolyA cassette, was designed. Cloning of the nDes-miR155- amiR-DMPK^{204}-minBGHpolyA cassette into the ITR plasmid was performed. The synthesized nDes-miR155- amiR-DMPK204-minBGHpolyA included a 5' Nco1 site and 3' Sph1 site for cloning into ITR plasmid (**FIG. 1B**). Briefly, the plasmid containing the synthesized nDes-miR155-amiR-DMPK2^{04}-minBGHpolyA sequence was digested with NcoI and SphI and the 1.9 kB fragment was gel purified. The ITR plasmid was digested with NcoI and SphI, dephosphorylated using Calf Intestinal Alkaline Phosphatase (New England Biolabs; Cat No M0290) and the 8.2 kB vector backbone fragment was gel purified. The digested 1.9 kB fragment containing the expression cassette and the digested ITR plasmid were ligated to produce plasmid ITR-nDes-miR155- amiR- DMPK^{204}.

Small-scale rAAV vector production

[0291] A small-scale packaging assay was performed in HEK 293 cells to confirm packaging of the ITR-nDes-miR155-amiR-DMPK^{204} plasmid. Small-scale production was performed using the AAV rep/cap plasmid. **FIG. 1C** shows the amount of vector produced per HEK 293 cell as compared to a standard EGFP plasmid gene cassette.

[0292] To determine the potential of amiR-DMPK^{204} to correct the DM1 phenotype, the ability to silence the human DMPK transcript and correct splicing defects in skeletal myoblast cultures from DM1 patients was tested. Due to the CTG expansion in the 3' UTR of the DMPK

gene, DMPK mRNA hairpin structures aggregate as insoluble ribonuclear foci and sequester several RNA-binding proteins. The resulting redistribution of essential splicing factors, such as muscleblind-like 1 (MBNL1), causes mis-splicing of downstream effectors responsible for the differentiation of muscle tissue. The treatment of DM1 patient cells with amiR-DMPK^{204} caused over 50% DMPK mRNA silencing and splicing correction, as measured by MBNL1 exon 7 inclusion.

Example 2: Processing of miR155 amiR-DMPK^{204}

[0293] The amiR-DMPK^{204} was packaged into an AAV capsid and DMPK knockdown efficacy, passenger strand activity, and processing patterns were analyzed *in vivo*. The constructs harboring nDes-miR155- amiR-DMPK^{204} were packaged into AAV. The vectors were intravenously injected into the DMSXL adult humanized DM1 mice model, which expresses human DMPK with >1,000 CTG repeats. After eight weeks, animals were euthanized, multiple tissues were collected to measure DMPK knockdown efficacy, and heart tissue was selected to measure the passenger strand activity and processing patterns.

[0294] RT-PCR analysis showed robust expression of amiR-DMPK^{204} in multiple muscle tissues with higher expression in the heart (**FIG. 2B**). Concomitant with amiR-DMPK^{204} expression, RT PCR analysis confirmed robust DMPK suppression in the heart with an average of >70% and ~30% DMPK suppression in different skeletal muscles (**FIG. 2C**). In heart tissue, DMPK expression is below 50% relative to TBP (TATA binding protein) expression. Notably, DSMXL mice expressing low levels of DMPK relative to TBP (~50%, indicated by the dotted line in **FIG. 2C**) do not have an obvious DM1 phenotype. Interestingly, the nDesmin promoter showed strong activity in the cardiac tissue and similar levels in skeletal muscle (**FIG. 2B**) even though higher transduction was observed in the liver **(FIG. 2A**). This suggests expression was mainly restricted to the cardiac and skeletal muscle, affected by DM1 pathology.

[0295] To assess the processing of the amiR-DMPK^{204}, heart tissues were analyzed for the mature amiR-DMPK^{204} lengths and sequence composition of the guide and passenger strands by NGS for small transcriptome analysis.

The processing of the amiR-DMPK^{204} did not produce passenger strands. amiR-DMPK^{204} was processed exclusively into guide strands (>99%) in mouse cardiomyocytes, but often produced longer stands than the predicted from the miRBase database (**Tables 1**). miR155 processing most often generated mature lengths between 22 and 26 nt long but processed accurately at 5' end **Table 1.** The sequence distributions of the different guide strand lengths (nt) mapping to miR155 amiR-DMPK^{204} calculated as percentages (% reads). The expected amiR-DMPK^{204} guide strand

is underlined and seed sequence is in Bold. The asterisk indicates reads corresponding to the predicted length of the amiR-DMPK^{204} guide strand.

Table 1. The sequence distributions of the different guide strand lengths (nt) mapping to miR155 amiR-DMPK^{204} calculated as percentages (% reads). The expected amiR-DMPK^{204} guide strand is underlined. The asterisk indicates reads corresponding to the predicted length of the amiR-DMPK^{204} guide strand.

miR-155 read sequence	Length	% Seqs	SEQ ID NO
A**GTCGAAG**ACAGTTCTAGGGTGT	23	52	33
A**GTCGAAG**ACAGTTCTAGGGTGTT	24	24	34
A**GTCGAAG**ACAGTTCTAGGGTGTTT	25	11	35
A**GTCGAAG**ACAGTTCTAGGGT*	22	6	36
A**GTCGAAG**ACAGTTCTAGGGTGTTTT	26	3	37
A**GTCGAAG**ACAGTTCTAGGGTG	22	4	38

[0296] Overall, no passenger strands were detected by the amiR-DMPK^{204} with miR155 (The % guide is > 99%). Therefore, miR155 was selected as the lead for the pre-clinical studies, as the miR155 miRNA scaffold is well-validated for RNAi.

Example 3: Dose Dependent suppression of human DMPK by systemic injection of AAV encoding miR155-amiRDMPK^{204} in Transgenic Mice

[0297] To determine the most efficacious dose, the delivery of three separate doses of myotropic AAV (WO/2019/207132) capsid was investigated. AAV encoding the expression cassette for amiR-DMPK^{204} with the miR155 scaffold (amiR155-DMPK^{204}) was evaluated in a dose-escalation study. Eight-week-old DMSXL mice were injected intravenously with 5.0×10^{11} vector genomes (vg)/kg, 5×10^{12} vg/kg and 1.0×10^{13} vg/kg, corresponding to low, intermediate, and high doses. Mice were analyzed for clinical symptoms such as body weight, survival, myotonia and cardiac function at 8 weeks following AAV infusion. Mice were euthanized 8 weeks post gene transfer, and DMPK suppression and splicing correction were measured. miR155-amiRDMPK^{204} expression levels were measured by small RNA TaqMan, and mRNA input levels was normalized to u6 small nuclear RNA.

[0298] The expression of amiR155-DMPK^{204} was observed in a dose-dependent manner (**FIG. 3A**) and resulted in a dose-dependent reduction of total DMPK expression (**FIG. 3B**) in multiple tissues. Overall, it was observed that ~10 amiR155-DMPK^{204} copies/U6 was sufficient

to reduce DMPK by ≥50% in the heart and diaphragm, an amount which may be sufficient to treat DM1 patients.

[0299] Next, the consequences of DMPK suppression on characteristic DM1 phenotypes, such as splicing abnormalities, was investigated. Aberrant splicing of LIM domain binding 3 (Ldb3), resulting in inclusion of exon 11 in Ldb3 transcripts, has been demonstrated as DM1-specific phenotype resulting from sequestration of RNA splicing machinery by CUG repeat RNA (Yamashita *et al.* 2014. *Neurobiol Dis.* 69:200-5). A significant reduction in the Ldb3 transcript with the inclusion of exon 11 in the gastrocnemius muscle was observed in mice that were treated with the medium-dose or the high dose of AAV nDes-miR155- amiR-DMPK^{204}, confirming that splicing defects were efficiently corrected in muscle treated with amiR155-DMPK^{204} (**FIG. 4**).

[0300] In addition to these molecular corrections, the efficacy of AAV nDes-miR155- amiR-DMPK^{204} was then measured in terms of physiological and functional manifestations of the disease.

[0301] To determine whether the treatment can improve survival and attenuate loss in body weight, DMSXL mice were treated with AAV nDes-miR155- amiR-DMPK^{204} at three different doses and monitored the survival and body weight. Improved body weight and survival rate were observed after eight weeks of treatment with medium and high doses. On the other hand, no improvement was observed with low dose or Balanced Salt Solution (BSS) control (**FIG. 5A** and **5B**).

[0302] Next, the efficacy of AAV nDes-miR155- amiR-DMPK^{204} was measured in terms of functional manifestations of disease such as prevention of myotonia and cardiac abnormalities. Electromyography measurements revealed a significant decrease in myotonia in mice treated with AAV nDes-miR155- amiR-DMPK^{204} (**Table 2**). In particular, after treatment, only 7.6% of the animals which were treated with the medium dose had myotonia. In contrast, >50% of mice in control (treated with BSS) or low dose groups had persistent myotonia (scores 1).

Table 2. Number of DMSXL DM1 mice with myotonia after treatment with AAV nDes-miR155- amiR-DMPK[204].

Group	Number of Animals with Myotonia (gastrocnemius)
WT	0/10
DMSXL-Ctrl (untreated)	4/7
DMSXL-5E11 VG/Kg (low dose)	3/5
DMSXL-5E12 VG/Kg (medium dose)	1/13
DMSXL-5E13 VG/Kg (high dose)	1/7

P<0.05

Myotonic discharges were graded 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.

[0303] Cardiac function of the DMSXL mice was also monitored using surface echocardiogram 8 weeks post-treatment along with the skeletal muscle function. AAV nDes-miR155- amiR-DMPK[204] improved cardiac output as compared to BSS treated controls. Significant improvement in cardiac output was noted in medium dose group (5e12 vg/kg) after 8 weeks of treatment (**FIG. 6**).

Example 4: The AAVrh74N502I capsid has improved muscle transduction and reduced liver transduction

[0304] An experiment was performed to test the transduction efficiency of AAV capsids containing the AAVrh74N502I VP1 capsid protein (WO2019178412; SEQ ID NO: 50) in various tissues in non-human primates. An outline of the experiment is shown in **FIG. 7A**. Non-human primates were treated intravenously with 1 x 10^{13} vg/kg of either AAV9, AAVrh74, or AAVrh74N502I capsids, each containing an eGFP expression cassette. Twenty-one days after treatment, the animals were sacrificed, and the levels of eGFP expression in the tibialis anterior (TA), bicep femoris, quadriceps, heart, and liver were measured.

[0305] The capsids containing the AAVrh74N502I capsid protein had improved muscle transduction (**FIGS. 7B-7E**) and reduced liver transduction (**FIG. 7F**) in the non-human primate compared to comparator capsids. (**Table 3**).

Table 3. Increase in eGFP levels in tissues of non-human primates treated with AAVrh74N502I capsids relative to those treated with AAV9 and AAVrh74 capsids.

Tissue	AAV9	AAVrh74
Tibialis Anterior	+13x	+178x
Bicep Femoris	+303x	+56x
Quadriceps	NS*	+32x
Heart	NS	+13x
Liver	-15x	-2x

*NS = not significant

Example 5: Evaluation of AAVrh74N502I nDes-miR155- amiR-DMPK^{204} target engagement in DMSXL mice model

[0306] To determine the most efficacious dose, the delivery of two separate doses of AAVrh74N502I capsid were investigated. AAV encoding the expression cassette for amiR-DMPK^{204} with the miR155 scaffold (miR155- amiR-DMPK^{204}) was evaluated in a dose-escalation study. Eight-week-old DMSXL mice were injected intravenously with 9×10^{13} vector genomes (vg)/kg, and 1.8×10^{14} vg/kg, corresponding to low, and high doses. Mice were euthanized 8 weeks post gene transfer, and DMPK suppression and amiR-DMPK^{204} expression levels were measured by small RNA TaqMan, and mRNA input levels was normalized to u6 small nuclear RNA.

[0307] The expression of amiR-DMPK^{204} was observed in a dose-dependent manner (**FIG. 8A**) and resulted in a dose-dependent reduction of total DMPK expression (**FIG. 8B**) in multiple tissues. Overall, it was observed that ~10 amiR-DMPK^{204} copies/U6 was sufficient to reduce DMPK by ≥50% in the heart and diaphragm, an amount which may be sufficient to treat DM1 patients.

[0308] Finally, the lead vector, nDes-miR155-amiR-DMPK^{204}, in the context of a myotropic capsid, AAVrh74N502I (SEQ ID NO: 50), was shown to have potent in vitro activity in the cardiomyocytes derived DM1 iPSCs **(FIG. 9).**

Example 6: Evaluation of AAVrh74N502I nDes-miR155- amiR-DMPK^{204} on transcriptome

[0309] To determine whether amiR-DMPK^{204} treatment had any major effect on the transcriptome, genome-wide RNA sequencing (RNA-seq) was performed, comparing amiR-DMPK^{204} -treated to CTL3 (scramble miRNA) by transfecting CBA miR155- amiR-DMPK^{204} plasmid into HEK293 cell lines. To evaluate whether the observed non-*DMPK* gene expression changes were due to the off-target effects of the amiR-DMPK^{204}, enrichment for seed complementarity in significantly downregulated targets was evaluated. There were four differentially expressed genes OPN4 (12.5 fold), DMPK (1.7 fold), KRTAP21-2 (1.7 fold), C8ORF44-SGK3 (1.7 fold) whose 3′ UTRs contain the TTCGAC seed complement, using a 5% false discovery rate (FDR) significance threshold (**Table 4**). With a 1% FDR, only OPN4 (12.5 fold), DMPK (1.7 fold), showing differential expression (**FIG. 10**). As expected, *DMPK* was one of the most significantly impacted mRNA levels (44% silencing). Overall, minimal off target effects were observed following over expression of amiR-DMPK^{204} in HEK293 cells.

Table 4

Gene Name	log2 fold 204 vs CTL3	Fold 204 vs CTL3	P value	FDR_BH <0.05	FDR_BH <0.01
OPN4	-3.64	12.5	2.7746E-17	5E-13	yes
DMPK	-0.77	1.7	3.4308E-10	2E-06	yes
KRTAP21-2	-0.76	1.7	0.0001	0.0103	
C8orf44-SGK3	-0.79	1.7	0.0003	0.0191	

Example 7: Dose range finding study to explore biodistribution and activity of AAVrh74N502I nDes-miR155- amiR-DMPK^{204} in non-human primates

To determine the biodistribution and activity of AAVrh74MN502I nDes-miR155- amiR-DMPK^{204}, a single intravenous infusion (IV) dose was administered to cynomolgus monkeys. The study duration was for 12 weeks after the single injection.

Sixteen total cynomolgous monkeys (8 male, 8 female; 24 to 48 months old) were dosed via IV in the saphenous vein once on day 1 of the study. Doses are indicated in Table 5 below. The animals were grouped (2 males and 2 females per group) into four different categories based on the dose level (vg/kg): formulation buffer (Group 1); 5 x 10^{13} vg/kg (Group 2); 1 x 10^{14} vg/kg

(Group 3); and 2 x 10^{14} vg/kg (Group 4). After 12 weeks, the animals were sacrificed and tissue was harvested for analysis.

Table 5

Group	Test Article or Control Article	Dose Level (vg/kg)	Dose Concentration (vg/mL)	Number of Animals	
				Males	Females
1	Formulation Buffer	0	0	2	2
2	AAVrh74N502I nDes-miR155- amiR-DMPK^{204}	5 x 10^{13}	5 x 10^{12} vg/ml	2	2
3	AAVrh74N502I nDes-miR155- amiR-DMPK^{204}	1 x 10^{14}	1 x 10^{13} vg/ml	2	2
4	AAVrh74N502I nDes-miR155- amiR-DMPK^{204}	2 x 10^{14}	2 x 10^{13} vg/ml	2	2

Harvested tissue was mechanically homogenized for RNA and DNA extraction, and samples were analyzed with digital PCR (dPCR).

Dose-dependent biodistribution and activity of AAVrh74N502I nDes-miR155- amiR-DMPK204 was demonstrated in several muscle and non-muscle tissues. Several skeletal muscles were analyzed (tibialis anterior muscle (TA); gastrocnemius; quadricep; biceps; soleus; extensor digitorum longus (EDL); diaphragm) as well as heart muscle and liver tissue. Viral genome copies were found in all tissues tested and the number of copies/cell in each tissue were in a dose-dependent manner (FIG. 12). The expression of amiR-DMPK expression (FIG. 13) and the downregulation of DMPK (FIG. 14) was also in dose-dependent manner in various muscle tissues, heart tissue, and liver tissue. The dose-dependent reduction of DMPK expression was found to be up to 90% reduced as compared to a control group. All doses tested in the animals were found to be safe and well-tolerated by the animals.

SEQUENCES

All polypeptide sequences are presented as N-terminal to C-terminal unless indicated otherwise.

All nucleic acid sequences are presented as 5' to 3' unless indicated otherwise.

Byrne desmin enhancer sequence

CACCCATGCCTCCTCAGGTACCCCCTGCCCCCCACAGCTCCTCTCCTGTGCCTTGTT
TC
CCAGCCATGCGTTCTCCTCTATAAATACCCGCTCTGGTATTTGGGGTTGGCAGCTGT
TG
CTGCCAGGGAGATGGTTGGGTTGACATGCGGCTCCTGACAAAACACAAACCCCTGGT
GT
GTGTGGGCGTGGGTGGTGTGAGTAGGGGGATGAATCAGGGAGGGGGCGGGGGACCCA
GGGGGCAGGAGCCACACAAAGTCTGTGCGGGGGTGGGAGCGCACATAGCAATTGGAA
ACTG
AAAGCTTATCAGACCCTTTCTGGAAATCAGCCCACTGTTTATAAACTTGAGGCCCCA
CCCTCGA **(SEQ ID NO:21)**

Source: Li and Paulin, et. al. 1991. "High level desmin expression depends on a muscle-specific enhancer." Journal of Biol Chem. 266.10: 6562-6570.

Homo sapiens desmin locus control region (DES-LCR) on chromosome 2 (NCBI Reference Sequence:NG_046330.1)

Byrne enhancer sequence corresponds to 17767-18125 in Ref Seq.

Paulin desmin enhancer sequence

CCCCCTGCCCCCCACAGCTCCTCTCCTGTGCCTTGTTTCCCAGCCATGCGTTCTCCT
CT
ATAAATACCCGCTCTGGTATTTGGGGTTGGCAGCTGTTGCTGCCAGGGAGATGGTTG
GG
TTGACATGCGGCTCCTGACAAAACACAAACCCCTGGTGTGTGTGGGCGTGGGTGGTG
TG
AGTAGGGGGATGAATCAGGGAGGGGGCGGGGGACCCAGGGGGCAGGAGCCACACAAA
GTCTGTGCGGGGGTGGGAGCGCACATAGCAATTGGAAACTGAA **(SEQ ID NO:22)**

Source: Li and Paulin, et. al. 1991. "High level desmin expression depends on a muscle-specific enhancer." Journal of Biol Chem. 266.10: 6562-6570.

Homo sapiens desmin locus control region (DES-LCR) on chromosome 2 (NCBI Reference Sequence:NG_046330.1)

Paulin enhancer sequence corresponds to 17787-18063 in Ref Seq

Paulin desmin promoter sequence (-228 to +75)

CTGCAGAC**C**TGCTTGCTGCCTGCCCTGGCGAAGGATTGGCAGGCTTGCCCGTCACAG
GA
CCCCCGCTGGCTGACTCAGGGGCGCAGGCCTCTTGCGGGGGAGCTGGCCTCCCCGCC

CC
CACGGCCACGGGCCGCCCTTTCCTGGCAGGACAGCGGGATCTTGCAGCTGTCAGGGG
AG
GGGAGGCGGGGGCTGATGTCAGGAGGGATACAAATAGTGCCGACGGCTGGGGGCCCT
GT
CTCCCCTCGCCGCATCCACTCTCCGGCCGGCCGCCTGCCCGCCGCCTCCTCCGTGCG
CCCGCCAGCCTCGCCCG **(SEQ ID NO:23)**

There is a one basepair difference from published sequence (C instead of an A, shown in bold and underlined).

Source: Li and Paulin, et. al. 1991. "High level desmin expression depends on a muscle-specific enhancer." Journal of Biol Chem. 266.10: 6562-6570.

Homo sapiens desmin locus control region (DES-LCR) on chromosome 2 (NCBI Reference Sequence:NG_046330.1)

Paulin promoter sequence corresponds to 18535- 18844 in Ref Seq

Complete nDes promoter sequence

CACCCATGCCTCCTCAGGTACCCCCTGCCCCCCACAGCTCCTCTCCTGTGCCTTGTT
TC
CCAGCCATGCGTTCTCCTCTATAAATACCCGCTCTGGTATTTGGGGTTGGCAGCTGT
TG
CTGCCAGGGAGATGGTTGGGTTGACATGCGGCTCCTGACAAAACACAAACCCCTGGT
GT
GTGTGGGCGTGGGTGGTGTGAGTAGGGGGATGAATCAGGGAGGGGGCGGGGGACCCA
GG
GGGCAGGAGCCACACAAAGTCTGTGCGGGGGTGGGAGCGCACATAGCAATTGGAAAC
TG
AAAGCTTATCAGACCCTTTCTGGAAATCAGCCCACTGTTTATAAACTTGAGGCCCCA
CCCTCGAGGTACCCCCTGCCCCCCACAGCTCCTCTCCTGTGCCTTGTTTCCCAGCCA
TGCG
TTCTCCTCTATAAATACCCGCTCTGGTATTTGGGGTTGGCAGCTGTTGCTGCCAGGG
AG
ATGGTTGGGTTGACATGCGGCTCCTGACAAAACACAAACCCCTGGTGTGTGTGGGCG
TG
GGTGGTGTGAGTAGGGGGATGAATCAGGGAGGGGGCGGGGGACCCAGGGGGCAGGAG
CC
ACACAAAGTCTGTGCGGGGGTGGGAGCGCACATAGCAATTGGAAACTGAAAGCTTCT
GC
AGACCTGCTTGCTGCCTGCCCTGGCGAAGGATTGGCAGGCTTGCCCGTCACAGGACC
CC
CGCTGGCTGACTCAGGGGCGCAGGCCTCTTGCGGGGGAGCTGGCCTCCCCGCCCCCA
CG
GCCACGGGCCGCCCTTTCCTGGCAGGACAGCGGGATCTTGCAGCTGTCAGGGGAGGG
GA
GGCGGGGGCTGATGTCAGGAGGGATACAAATAGTGCCGACGGCTGGGGGCCCTGTCT
CC
CCTCGCCGCATCCACTCTCCGGCCGGCCGCCTGCCCGCCGCCTCCTCCGTGCGCCCG
CCAGCCTCGCCCG **(SEQ ID NO:12)**

Source: Li and Paulin, et. al. 1991. "High level desmin expression depends on a muscle-specific enhancer." Journal of Biol Chem. 266.10: 6562-6570.

Rabbit β-globin intron

GTGAGTTTGGGGACCCTTGATTGTTCTTTCTTTTTCGCTATTGTAAAATTCATGTTATA
TGGAGGGGGCAAAGTTTTCAGGGTGTTGTTTAGAATGGGAAGATGTCCCTTGTATCACC
ATG**CATG**GACCCTCATGATAATTTTGTTTCTTTCACTTTCTACTCTGTTGACAACCATT
GTCTCCTCTTATTTTCTTTTCATTTTCTGTAACTTTTTCGTTAAACTTTAGCTTGCATT
TGTAACGAATTTTTAAATTCACTTTTGTTTATTTGTCAGATTGTAAGATCCCATCGATT
CCAATCAGGGTATATTATATTGTACTTCAGCACAGTTTTAGAGAACAATTGTTATAATTA
AATGATAAGGTAGAATATTTCTGCATATAAATTCTGGCTGGCGTGGAAATATTCTTATT
GGTAGAAACAACTACA**T**CCTGGTCATCATCCTGCCTTTCTCTTTATGGTTACAATGATA
TACACTGTTTGAGATGAGGATAAAATACTCTGAGTCCAAACCGGGCCCCTCTGCTAACC
ATGTTCATGCCTTCTTCT**T**TTTCCTACAG **(SEQ ID NO: 13)**

5' arm of rabbit β-globin intron

GTGAGTTTGGGGACCCTTGATTGTTCTTTCTTTTTCGCTATTGTAAAATTCATGTTA
TA
TGGAGGGGGCAAAGTTTTCAGGGTGTTGTTTAGAATGGGAAGATGTCCCTTGTATCA
CC
ATG**CATG**GACCCTCATGATAATTTTGTTTCTTTCACTTTCTACTCTGTTGACAACCA
TT
GTCTCCTCTTATTTTCTTTTCATTTTCTGTAACTTTTTCGTTAAACTTTAGCTTGCA
TT
TGTAACGAATTTTTAAATTCACTTTTGTTTATTTGTCAGATTGTAAGATCCCATCGA
TTC **(SEQ ID NO:14)**

Source: *Oryctolagus cuninculus* hemoglobin, beta (HBB2) Gene ID 100009084

The sequence of the present invention has an additional CATG (shown in bold and underlined) that is not present in Gene ID 100009084.

3' arm of rabbit β-globin intron

CAATCAGGGTATATTATATTGTACTTCAGCACAGTTTTAGAGAACAATTGTTATAAT
TA
AATGATAAGGTAGAATATTTCTGCATATAAATTCTGGCTGGCGTGGAAATATTCTTA
TT
GGTAGAAACAACTACA**T**CCTGGTCATCATCCTGCCTTTCTCTTTATGGTTACAATGA
TA
TACACTGTTTGAGATGAGGATAAAATACTCTGAGTCCAAACCGGGCCCCTCTGCTAA
CCATGTTCATGCCTTCTTCT**T**TTTCCTACAG **(SEQ ID NO:15)**

Source: Oryctolagus cuninculus hemoglobin, beta (HBB2) Gene ID 100009084

The sequence of the present invention has two T residues (shown in bold and underlined above) instead of two C residues in the Gene ID 100009084.

miR155-DMPK^{204} Sequences:

5' miR155 flanking sequence

RNA sequence
CUGGAGGCUUGCUGAAGGCUGUAUGCU (**SEQ ID NO:9**)

DNA sequence
CTGGAGGCTTGCTGAAGGCTGTATGCT (**SEQ ID NO:40**)
Source: BLOCK-iT™ Pol II miR RNAi Expression Vector Kits catalog #K493500

The engineered pre-miRNA sequence structure is based on the murine miR-155 sequence (Lagos-Quintana *et al.*, 2002, *Current Biology*, 12:9, 735-739).

amiR-DMPK^{204} Guide-DNA
AGTCGAAGACAGTTCTAGGGT (**SEQ ID NO:4**)

miR155 terminal loop-DNA
GTTTTGGCCACTGACTGAC (**SEQ ID NO:6**)

Source: BLOCK-iT™ Pol II miR RNAi Expression Vector Kits catalog #K493500

The engineered pre-miRNA sequence structure is based on the murine miR-155 sequence (Lagos-Quintana *et al.*, 2002, *Current Biology*, 12:9, 735-739).

amiR-DMPK^{204} Passenger-DNA
ACCCTAGATGTCTTCGATT (**SEQ ID NO:5**)

amiR-DMPK^{204} Guide-RNA- miR155 terminal loop- amiR-DMPK^{204} Passenger-RNA
AGUCGAAGACAGUUCUAGGGUUGUUUUGGCCACUGACUGACACCCUAGAUGUCUUCGAUU
(**SEQ ID NO:7**)

amiR-DMPK^{204} Guide-DNA- miR155 terminal loop- amiR-DMPK^{204} Passenger-DNA
AGTCGAAGACAGTTCTAGGGTTGTTTTGGCCACTGACTGACACCCTAGATGTCTTCGATT
(**SEQ ID NO:8**)

3' miR155 flanking sequence
RNA sequence
GACACAAGGCCUGUUACUAGCACUCACAUGGAACAAAUGGCC (**SEQ ID NO:10**)

DNA sequence
GACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCC (**SEQ ID NO:41**)

Source: BLOCK-iT™ Pol II miR RNAi Expression Vector Kits catalog #K493500

The engineered pre-miRNA sequence structure is based on the murine miR-155 sequence (Lagos-Quintana *et al.*, 2002, *Current Biology*, 12:9, 735-739).

miR155

RNA
CUGGAGGCUUGCUGAAGGCUGUAUGCUGACACAAGGCCUGUUACUAGCACUCACAUG
GAACAAAUGGCC **(SEQ ID NO:11)**

DNA
CTGGAGGCTTGCTGAAGGCTGTATGCTGACACAAGGCCTGTTACTAGCACTCACATG
GAACAAATGGCC **(SEQ ID NO:42)**

Full miR155-DMPK^{204} duplex sequence - RNA

CUGGAGGCUUGCUGAAGGCUGUAUGCUGAGUCGAAGACAGUUCUAGGGUGUUUUGGC
CACUGACUGACACCCUAGAUGUCUUCGAUUCAGGACACAAGGCCUGUUACUAGCACU
CACAUGGAACAAAUGGCC **(SEQ ID NO:24)**

Full miR155-DMPK^{204} duplex sequence - DNA
CTGGAGGCTTGCTGAAGGCTGTATGCTGAGTCGAAGACAGTTCTAGGGTGTTTTGGC
CACTGACTGACACCCTAGATGTCTTCGATTCAGGACACAAGGCCTGTTACTAGCACT
CACATGGAACAAATGGCC **(SEQ ID NO:25)**

minimal BGHpA sequence

TCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGG
TGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTA
GGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAA
GACAATAGC **(SEQ ID NO:16)**

Source: *Bos taurus* growth hormone 1 (GH1) mRNA NCBI Ref Seq NM_180996.1

1138 bp A1AT intron stuffer sequence (upstream of expression cassette)

TACGTACAATTGGGATCCTTCGAACTTGAGAGAAAACATCCCAGGGATTTACAGATC
AC
ATGCAGGCAGGGACCAGCTCAACCCTTCTTTAATGTCATCCAGGGAGGGGGCCAGGG
AT
GGAGGGGAGGGGTTGAGGAGCGAGAGGCAGTTATTTTTGGGTGGGATTCACCACTTT
TC
CCATGAAGAGGGGAGACTTGGTATTTTGTTCAATCATTAAGAAGACAAAGGGTTTGT
TG
AACTTGACCTCGGGGGGGATAGACATGGGTATGGCCTCTAAAAACATGGCCCCAGCA
GC
TTCAGTCCCTTTCTCGTCGATGGTCAGCACAGCCTTATGCACGGCCTGGAGGGGAGA
GA
AGCAGAGACACGTTGTAAGGCTGATCCCAGGCCTCGAGCAAGGCTCACGTGGACACC
TC
CCAGGAAGCGCTCACTCCCCCTGGACGGCCCTGGCCCTGCACATCCTCTCCCTCCCT

GT
CACATAGGCCTTGCTCCTCCTCAAGGCTTTGGCTGATGGGGCTGGCTCCCCTCTGTC
CA
TCTTCCTGACAAGCGCCTCTCCCCCTGCTCAGGTGCACCCACAACTCAGAACAGGGA
AG
AGCATCGTCACTCCACTAGTCTGCCTCCAGGGCTCTCTCCTTTCTAGTACACGGCTT
GA
AGCTCCTTGAGGACACGGACCCTGGCAGTGACCTTCACAGTGCCCAGACCCCAAGAT
AA
TGCAGCCATTCATGGAACTGCAGGTTGTTCATTGGTCGCCTTTAGTTTTCCAAAATA
AG
TGTCACTTTAGCTGAAATCATTCATTAATTCAGACACCAAATCTCACAGATCGAAGG
AG
TCAGAAATTCCTTTGAAACAACTTAGCCCAAACCTTTCTGTGTCAGTATGGATAAAT
CA
AGGCCCAATGTCTAGAAGGTCTTGGGCAAAGTTGAAATTCAGGGTCAGTGACACAAC
CT
CAAGGGAGGCCCCGAAAGTGCCAGCTGCACAGCAGCCCCTGCCTGGCTTTGCTGTTT
GC
CCACCGTCCCGTGTCAGTGAATCACGGGCATCTTCAGGAGCTCAGCCTGGGTCTTCA
TT
TGTTTCCCTCGGCCCCTTCCTCAGCCTCAGGACAGTGCTAGCAGCCCCCACACATTC
TTCCCTACAGATACCATGG **(SEQ ID NO:18)**

Source: plasmid DC 969 (SerpinA1= A1AT) chromosome 14 NG_008290.1

398 bp A1AT intron stuffer sequence (downstream of expression cassette)

GCATGCAGAGTGGACAGGGGCCTCAGGGACCCCTGATCCCAGCTTTCTCATTGGACA
GA
AGGAGGAGACTGGGGCTGGAGAGGGACCTGGGCCCCCACTAAGGCCACAGCAGAGCC
AG
GACTTTAGCTGTGCTGACTGCAGCCTGGCTTGCCTCCACTGCCCTCCTTTGCCTCAA
GA
GCAAGGGAGCCTCAGAGTGGAGGAAGCAGCCCCTGGCCTTGCCTCCCACCTCCCCTC
CC
CTATGCTGTTTTCCTGGGACAGTGGGAGCTGGCTTAGAATGCCCTGGGGCCCCCAGG
AC
CCTGGCATTTTAACCCCTCAGGGGCAGGAAGGCAGCCTGAGATACAGAAGAGTCCAT
CA CCTGCTGTATGCCACACACCATCCCCACAGTCGACATTTAAATT **(SEQ ID NO:19)**

Source: plasmid DC 969 (SerpinA1= A1AT) chromosome 14 NG_008290.1

ITR-nDes-miR155- amiR-DMPK^{204} -BGHpA-stuffer-ITR (3739 bp)

**TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGC
GAC**

CTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGG
GTT
CCTTACGTACAATTGGGATCCTTCGAACTTGAGAGAAAACATCCCAGGGATTTACAGATCACATGCAG
GCA
GGGACCAGCTCAACCCTTCTTTAATGTCATCCAGGGAGGGGGCCAGGGATGGAGGGGAGGGGTTGAGG
AGC
GAGAGGCAGTTATTTTTGGGTGGGATTCACCACTTTTCCCATGAAGAGGGGAGACTTGGTATTTTGTT
CAA
TCATTAAGAAGACAAAGGGTTTGTTGAACTTGACCTCGGGGGGGATAGACATGGGTATGGCCTCTAAA
AAC
ATGGCCCCAGCAGCTTCAGTCCCTTTCTCGTCGATGGTCAGCACAGCCTTATGCACGGCCTGGAGGGG
AGA
GAAGCAGAGACACGTTGTAAGGCTGATCCCAGGCCTCGAGCAAGGCTCACGTGGACACCTCCCAGGAA
GCG
CTCACTCCCCCTGGACGGCCCTGGCCCTGCACATCCTCTCCCTCCCTGTCACATAGGCCTTGCTCCTC
CTC
AAGGCTTTGGCTGATGGGGCTGGCTCCCCTCTGTCCATCTTCCTGACAAGCGCCTCTCCCCCTGCTCA
GGT
GCACCCACAACTCAGAACAGGGAAGAGCATCGTCACTCCACTAGTCTGCCTCCAGGGCTCTCTCCTTT
CTA
GTACACGGCTTGAAGCTCCTTGAGGACACGGACCCTGGCAGTGACCTTCACAGTGCCCAGACCCCAAG
ATA
ATGCAGCCATTCATGGAACTGCAGGTTGTTCATTGGTCGCCTTTAGTTTTCCAAAATAAGTGTCACTT
TAG
CTGAAATCATTCATTAATTCAGACACCAAATCTCACAGATCGAAGGAGTCAGAAATTCCTTTGAAACA
ACT
TAGCCCAAACCTTTCTGTGTCAGTATGGATAAATCAAGGCCCAATGTCTAGAAGGTCTTGGGCAAAGT
TGA
AATTCAGGGTCAGTGACACAACCTCAAGGGAGGCCCCGAAAGTGCCAGCTGCACAGCAGCCCCTGCCT
GGC
TTTGCTGTTTGCCCACCGTCCCGTGTCAGTGAATCACGGGCATCTTCAGGAGCTCAGCCTGGGTCTTC
ATT
TGTTTCCCTCGGCCCCTTCCTCAGCCTCAGGACAGTGCTAGCAGCCCCCACACATTCTTCCCTACAGA
TAC
CATGGCACCCATGCCTCCTCAGGTACCCCCTGCCCCCCACAGCTCCTCTCCTGTGCCTTGTTTCCCAG
CCA
TGCGTTCTCCTCTATAAATACCCGCTCTGGTATTTGGGGTTGGCAGCTGTTGCTGCCAGGGAGATGGT
TGG
GTTGACATGCGGCTCCTGACAAAACACAAACCCCTGGTGTGTGTGGGCGTGGGTGGTGTGAGTAGGGG
GAT
GAATCAGGGAGGGGGCGGGGGACCCAGGGGGCAGGAGCCACACAAAGTCTGTGCGGGGGTGGGAGCGC
ACA
TAGCAATTGGAAACTGAAAGCTTATCAGACCCTTTCTGGAAATCAGCCCACTGTTTATAAACTTGAGG
CCC
CACCCTCGAGGTACCCCCTGCCCCCCACAGCTCCTCTCCTGTGCCTTGTTTCCCAGCCATGCGTTCTC
CTC
TATAAATACCCGCTCTGGTATTTGGGGTTGGCAGCTGTTGCTGCCAGGGAGATGGTTGGGTTGACATG
CGG
CTCCTGACAAAACACAAACCCCTGGTGTGTGTGGGCGTGGGTGGTGTGAGTAGGGGGATGAATCAGGG
AGG
GGGCGGGGGACCCAGGGGGCAGGAGCCACACAAAGTCTGTGCGGGGGTGGGAGCGCACATAGCAATTG
GAA
ACTGAAAGCTTCTGCAGACCTGCTTGCTGCCTGCCCTGGCGAAGGATTGGCAGGCTTGCCCGTCACAG
GAC
CCCCGCTGGCTGACTCAGGGGCGCAGGCCTCTTGCGGGGGAGCTGGCCTCCCCGCCCCCACGGCCACG
GGC
CGCCCTTTCCTGGCAGGACAGCGGGATCTTGCAGCTGTCAGGGGAGGGGAGGCGGGGGCTGATGTCAG
GAG
GGATACAAATAGTGCCGACGGCTGGGGGCCCTGTCTCCCCTCGCCGCATCCACTCTCCGGCCGGCCGC
CTG
CCCGCCGCCTCCTCCGTGCGCCCGCCAGCCTCGCCCG*GAGCTCTGAGTAGACGAAGCTAAGGCGCGC*C
TGA
GAACTTCAGGGTGAGTTTGGGGACCCTTGATTGTTCTTTCTTTTTCGCTATTGTAAAATTCATGTTAT

ATG
GAGGGGGCAAAGTTTTCAGGGTGTTGTTTAGAATGGGAAGATGTCCCTTGTATCACCATGCATGGACC
CTC
ATGATAATTTTGTTTCTTTCACTTTCTACTCTGTTGACAACCATTGTCTCCTCTTATTTTCTTTTCAT
TTT
CTGTAACTTTTTCGTTAAACTTTAGCTTGCATTTGTAACGAATTTTTAAATTCACTTTTGTTTATTTG
TCA
GATTGTAAGATCCCATCGATTCGGATCCCTGGAGGCTTGCTGAAGGCTGTATGCTGAGTCGAAGACAG
TTC
TAGGGTGTTTTGGCCACTGACTGACACCCTAGATGTCTTCGATTCAGGACACAAGGCCTGTTACTAGC
ACT
CACATGGAACAAATGGCCCTCGAGCAATCAGGGTATATTATATTGTACTTCAGCACAGTTTTAGAGAA
CAA
TTGTTATAATTAAATGATAAGGTAGAATATTTCTGCATATAAATTCTGGCTGGCGTGGAAATATTCTT
ATT
GGTAGAAACAACTACATCCTGGTCATCATCCTGCCTTTCTCTTTATGGTTACAATGATATACACTGTT
TGA
GATGAGGATAAAATACTCTGAGTCCAAACCGGGCCCCTCTGCTAACCATGTTCATGCCTTCTTCTTTT
TCC
TACAGCTCCTGGGCAACGTGCTG*ACCGGT*CTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGC
CTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCAT
TGTCTG
AGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAA
TAGCGCATGCAGAGTGGACAGGGGCCTCAGGGACCCCTGATCCCAGCTTTCTCATTGGACAGAAGGAG
GAGACTGGGGCTGGAGAGGGACCTGGGCCCCCACTAAGGCCACAGCAGAGCCAGGACTTTAGCTGTGC
TGACTGCAG
CCTGGCTTGCCTCCACTGCCCTCCTTTGCCTCAAGAGCAAGGGAGCCTCAGAGTGGAGGAAGCAGCCC
CTG
GCCTTGCCTCCCACCTCCCCTCCCCTATGCTGTTTTCCTGGGACAGTGGGAGCTGGCTTAGAATGCCC
TGG
GGCCCCCAGGACCCTGGCATTTTAACCCCTCAGGGGCAGGAAGGCAGCCTGAGATACAGAAGAGTCCA
TCA
CCTGCTGTATGCCACACACCATCCCCACAGTCGACATTTAAATT**AGGAACCCCTAGTGATGGAGTTGG**
CCA
CTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTT
GGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAA

(SEQ ID NO:20)

Table 5. ITR-nDes-miR155- amiR-DMPK[204] Sequence Annotation from 5'ITR to 3'ITR (3739 bp)

Sequence Numbering	SEQ ID NO	Annotation
1 - 145	43	5' ITR
146 - 1283	44	A1AT stuffer
1284 - 2238	12	nDes promoter (details below)
1284 - 1642	21	Byrne enhancer
1643 - 1648	n/a	Kpn1 restriction site
1647 - 1923	22	Paulin enhancer
1923 - 1928	n/a	HindIII restriction site
1929 - 1934	n/a	PstI restriction site
1929 - 2238	23	Paulin promoter

Sequence Numbering	SEQ ID NO	Annotation
2268 - 2578	45	5' arm rabbit Bglobin intron
2579 - 2584	n/a	BamH1 restriction site
2585 - 2611	46	5'miR155 flanking sequence
2612	n/a	additional base from Invitrogen Block-IT kit
2613 - 2633	4	$DMPK^{204}$ miRNA guide
2634 - 2652	6	miR155 terminal loop
2653 - 2671	5	$DMPK^{204}$ miRNA passenger
2672 - 2674		additional base from Invitrogen Block-IT kit
2675 - 2716	41	3'miR155 flanking sequence
2717 - 2722	n/a	Xho1 restriction site
2723 - 3005	47	3' arm rabbit Bglobin intron
3011 - 3196	16	minBGH polyA
3197 – 3594	48	A1AT stuffer
3595 – 3739	49	3'ITR

Sequence of nDes-miR155-204 fragment synthesized:

AGATCTCCATGGCACCCATGCCTCCTCAGGTACCCCCTGCCCCCCACAGCTCCTCTCCTGTGCCTTGT
TTC
CCAGCCATGCGTTCTCCTCTATAAATACCCGCTCTGGTATTTGGGGTTGGCAGCTGTTGCTGCCAGGG
AGA
TGGTTGGGTTGACATGCGGCTCCTGACAAAACACAAACCCCTGGTGTGTGTGGGCGTGGGTGGTGTGA
GTA
GGGGGATGAATCAGGGAGGGGGCGGGGGACCCAGGGGGCAGGAGCCACACAAAGTCTGTGCGGGGGTG
GGA
GCGCACATAGCAATTGGAAACTGAAAGCTTATCAGACCCTTTCTGGAAATCAGCCCACTGTTTATAAA
CTT
GAGGCCCCACCCTCGAGGTACCCCCTGCCCCCCACAGCTCCTCTCCTGTGCCTTGTTTCCCAGCCATG
CGT
TCTCCTCTATAAATACCCGCTCTGGTATTTGGGGTTGGCAGCTGTTGCTGCCAGGGAGATGGTTGGGT
TGA
CATGCGGCTCCTGACAAAACACAAACCCCTGGTGTGTGTGGGCGTGGGTGGTGTGAGTAGGGGGATGA
ATC
AGGGAGGGGGCGGGGGACCCAGGGGGCAGGAGCCACACAAAGTCTGTGCGGGGGTGGGAGCGCACATA
GCA
ATTGGAAACTGAAAGCTTCTGCAGACCTGCTTGCTGCCTGCCCTGGCGAAGGATTGGCAGGCTTGCCC
GTC
ACAGGACCCCCGCTGGCTGACTCAGGGGCGCAGGCCTCTTGCGGGGGAGCTGGCCTCCCCGCCCCCAC
GGC
CACGGGCCGCCCTTTCCTGGCAGGACAGCGGGATCTTGCAGCTGTCAGGGGAGGGGAGGCGGGGGCTG
ATG
TCAGGAGGGATACAAATAGTGCCGACGGCTGGGGGCCCTGTCTCCCCTCGCCGCATCCACTCTCCGGC
CGG
CCGCCTGCCCGCCGCCTCCTCCGTGCGCCCGCCAGCCTCGCCCGGAGCTCTGAGTAGACGAAGCTAAG
GCG

CGCCTGAGAACTTCAGGGTGAGTTTGGGGACCCTTGATTGTTCTTTCTTTTTCGCTATTGTAAAATTC
ATG
TTATATGGAGGGGGCAAAGTTTTCAGGGTGTTGTTTAGAATGGGAAGATGTCCCTTGTATCACCATGC
ATG
GACCCTCATGATAATTTTGTTTCTTTCACTTTCTACTCTGTTGACAACCATTGTCTCCTCTTATTTTC
TTT
TCATTTTCTGTAACTTTTTCGTTAAACTTTAGCTTGCATTTGTAACGAATTTTTAAATTCACTTTTGT
TTA
TTTGTCAGATTGTAAGATCCCATCGATTCGGATCCCTGGAGGCTTGCTGAAGGCTGTATGCTGAGTCG
AAG
ACAGTTCTAGGGTGTTTTGGCCACTGACTGACACCCTAGATGTCTTCGATTCAGGACACAAGGCCTGT
TAC
TAGCACTCACATGGAACAAATGGCCCTCGAGCAATCAGGGTATATTATATTGTACTTCAGCACAGTTT
TAG
AGAACAATTGTTATAATTAAATGATAAGGTAGAATATTTCTGCATATAAATTCTGGCTGGCGTGGAAA
TAT
TCTTATTGGTAGAAACAACTACATCCTGGTCATCATCCTGCCTTTCTCTTTATGGTTACAATGATATA
CAC
TGTTTGAGATGAGGATAAAATACTCTGAGTCCAAACCGGGCCCCTCTGCTAACCATGTTCATGCCTTC
TTC
TTTTTCCTACAGCTCCTGGGCAACGTGCTGACCGGTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCC
CCC
GTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATC
GCA
TTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGG
AAGACAATAGCGCATGCGTCGACT **(SEQ ID NO: 26)**

nDes-miR155-204 promoter to polyA

CACCCATGCCTCCTCAGGTACCCCCTGCCCCCCACAGCTCCTCTCCTGTGCCTTGTTTC
CCAGCCATGCGTTCTCCTCTATAAATACCCGCTCTGGTATTTGGGGTTGGCAGCTGTTGCTGCCAGGGAGA
TGGTTGGGTTGACATGCGGCTCCTGACAAAACACAAACCCCTGGTGTGTGTGGGCGTGGGTGGTGTGAGTA
GGGGGATGAATCAGGGAGGGGGCGGGGGACCCAGGGGGCAGGAGCCACACAAAGTCTGTGCGGGGGTGGGA
GCGCACATAGCAATTGGAAACTGAAAGCTTATCAGACCCTTTCTGGAAATCAGCCCACTGTTTATAAACTT
GAGGCCCCACCCTCGAGGTACCCCCTGCCCCCCACAGCTCCTCTCCTGTGCCTTGTTTCCCAGCCATGCGT
TCTCCTCTATAAATACCCGCTCTGGTATTTGGGGTTGGCAGCTGTTGCTGCCAGGGAGATGGTTGGGTTGA
CATGCGGCTCCTGACAAAACACAAACCCCTGGTGTGTGTGGGCGTGGGTGGTGTGAGTAGGGGGATGAATC
AGGGAGGGGGCGGGGGACCCAGGGGGCAGGAGCCACACAAAGTCTGTGCGGGGGTGGGAGCGCACATAGCA
ATTGGAAACTGAAAGCTTCTGCAGACCTGCTTGCTGCCTGCCCTGGCGAAGGATTGGCAGGCTTGCCCGTC
ACAGGACCCCCGCTGGCTGACTCAGGGGCGCAGGCCTCTTGCGGGGGGAGCTGGCCTCCCCGCCCCCACGGC
CACGGGCCGCCCTTTCCTGGCAGGACAGCGGGATCTTGCAGCTGTCAGGGGAGGGGAGGCGGGGGCTGATG
TCAGGAGGGATACAAATAGTGCCGACGGCTGGGGGCCCTGTCTCCCCTCGCCGCATCCACTCTCCGGCCGG
CCGCCTGCCCGCCGCCTCCTCCGTGCGCCCGCCAGCCTCGCCCGGAGCTCTGAGTAGACGAAGCTAAGGCG
CGCCTGAGAACTTCAGGGTGAGTTTGGGGACCCTTGATTGTTCTTTCTTTTTCGCTATTGTAAAATTCATG
TTATATGGAGGGGGCAAAGTTTTCAGGGTGTTGTTTAGAATGGGAAGATGTCCCTTGTATCACCATGCATG
GACCCTCATGATAATTTTGTTTCTTTCACTTTCTACTCTGTTGACAACCATTGTCTCCTCTTATTTTCTTT
TCATTTTCTGTAACTTTTTCGTTAAACTTTAGCTTGCATTTGTAACGAATTTTTAAATTCACTTTTGTTTA
TTTGTCAGATTGTAAGATCCCATCGATTCGGATCCCTGGAGGCTTGCTGAAGGCTGTATGCTGAGTCGAAG
ACAGTTCTAGGGTGTTTTGGCCACTGACTGACACCCTAGATGTCTTCGATTCAGGACACAAGGCCTGTTAC
TAGCACTCACATGGAACAAATGGCCCTCGAGCAATCAGGGTATATTATATTGTACTTCAGCACAGTTTTAG
AGAACAATTGTTATAATTAAATGATAAGGTAGAATATTTCTGCATATAAATTCTGGCTGGCGTGGAAATAT
TCTTATTGGTAGAAACAACTACATCCTGGTCATCATCCTGCCTTTCTCTTTATGGTTACAATGATATACAC
TGTTTGAGATGAGGATAAAATACTCTGAGTCCAAACCGGGCCCCTCTGCTAACCATGTTCATGCCTTCTTC
TTTTTCCTACAGCTCCTGGGCAACGTGCTGACCGGTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCC
GTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCA
TTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAG
ACAATAGC

(SEQ ID NO: 17)

Amino Acid Sequence of an AAVrh74 variant (WO2019178412)

```
Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1               5                  10                  15
Glu Gly Ile Arg Glu Trp Trp Asp Leu Lys Pro Gly Ala Pro Lys Pro
            20                  25                  30
Lys Ala Asn Gln Gln Lys Gln Asp Asn Gly Arg Gly Leu Val Leu Pro
        35                  40                  45
Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
    50                  55                  60
Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65                  70                  75                  80
Gln Gln Leu Gln Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
                85                  90                  95
Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
            100                 105                 110
Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
        115                 120                 125
Leu Gly Leu Val Glu Ser Pro Val Lys Thr Ala Pro Gly Lys Lys Arg
    130                 135                 140
Pro Val Glu Pro Ser Pro Gln Arg Ser Pro Asp Ser Ser Thr Gly Ile
145                 150                 155                 160
Gly Lys Lys Gly Gln Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln
                165                 170                 175
Thr Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Ile Gly Glu Pro
            180                 185                 190
Pro Ala Gly Pro Ser Gly Leu Gly Ser Gly Thr Met Ala Ala Gly Gly
        195                 200                 205
Gly Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Ser
    210                 215                 220
Ser Ser Gly Asn Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val
225                 230                 235                 240
Ile Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His
                245                 250                 255
Leu Tyr Lys Gln Ile Ser Asn Gly Thr Ser Gly Gly Ser Thr Asn Asp
            260                 265                 270
Asn Thr Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn
        275                 280                 285
Arg Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn
    290                 295                 300
Asn Asn Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn
305                 310                 315                 320
Ile Gln Val Lys Glu Val Thr Gln Asn Glu Gly Thr Lys Thr Ile Ala
                325                 330                 335
Asn Asn Leu Thr Ser Thr Ile Gln Val Phe Thr Asp Ser Glu Tyr Gln
            340                 345                 350
Leu Pro Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe
        355                 360                 365
Pro Ala Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn
    370                 375                 380
Asn Gly Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr
385                 390                 395                 400
Phe Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Glu Phe Ser Tyr
                405                 410                 415
Asn Phe Glu Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser
            420                 425                 430
Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu
        435                 440                 445
Ser Arg Thr Gln Ser Thr Gly Gly Thr Ala Gly Thr Gln Gln Leu Leu
    450                 455                 460
Phe Ser Gln Ala Gly Pro Asn Asn Met Ser Ala Gln Ala Lys Asn Trp
465                 470                 475                 480
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Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Thr Thr Leu Ser
                485                 490                 495
Gln Asn Asn Asn Ser Asn Phe Ala Trp Thr Gly Ala Thr Lys Tyr His
            500                 505                 510
Leu Asn Gly Arg Asp Ser Leu Val Asn Pro Gly Val Ala Met Ala Thr
        515                 520                 525
His Lys Asp Asp Glu Glu Arg Phe Phe Pro Ser Ser Gly Val Leu Met
    530                 535                 540
Phe Gly Lys Gln Gly Ala Gly Lys Asp Asn Val Asp Tyr Ser Ser Val
545                 550                 555                 560
Met Leu Thr Ser Glu Glu Glu Ile Lys Thr Thr Asn Pro Val Ala Thr
                565                 570                 575
Glu Gln Tyr Gly Val Val Ala Asp Asn Leu Gln Gln Gln Asn Tyr Ile
            580                 585                 590
Gly Ser Arg Gly Ala Val Asn Ser Gln Gly Ala Leu Pro Gly Met Val
        595                 600                 605
Trp Gln Asn Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile
    610                 615                 620
Pro His Thr Asp Gly Asn Phe His Pro Ser Pro Leu Met Gly Gly Phe
625                 630                 635                 640
Gly Leu Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val
                645                 650                 655
Pro Ala Asp Pro Pro Thr Thr Phe Asn Gln Ala Lys Leu Ala Ser Phe
            660                 665                 670
Ile Thr Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu
        675                 680                 685
Leu Gln Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr
    690                 695                 700
Ser Asn Tyr Tyr Lys Ser Thr Asn Val Asp Phe Ala Val Asn Thr Glu
705                 710                 715                 720
Gly Thr Tyr Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg
                725                 730                 735

Asn Leu
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(SEQ ID NO:39)

Amino Acid Sequence of AAVrh74N502I

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDNGRGLVLPGYKYLGPFN
GLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGNL
GRAVFQAKKRVLEPLGLVESPVKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPAKKRLN
FGQTGDSESVPDPQPIGEPPAGPSGLGSGTMAAGGGAPMADNNEGADGVGSSSGNWHC
DSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGSTNDNTYFGYSTPWGYFDFNRF
HCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFTDSE
YQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTG
NNFEFSYNFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQSTGGTAGTQQLLFSQAG
PNNMSAQAKNWLPGPCYRQQRVSTTLSQNNNSIFAWTGATKYHLNGRDSLVNPGVAM
ATHKDDEERFFPSSGVLMFGKQGAGKDNVDYSSVMLTSEEEIKTTNPVATEQYGVVAD
NLQQQNAAPIVGAVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGL
KHPPPQILIKNTPVPADPPTTFNQAKLASFITQYSTGQVSVEIEWELQKENSKRWNPEIQY
TSNYYKSTNVDFAVNTEGTYSEPRPIGTRYLTRNL (**SEQ ID NO: 50**)

Nucleotide Sequence encoding AAVrh74 N502I capsid

ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTCTCTGAGGGCATT
CGCGAGTGGTGGGACCTGAAACCTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAA
GCAGGACAACGGCCGGGGTCTGGTGCTTCCTGGCTACAAGTACCTCGGACCCTTCAA
CGGACTCGACAAGGGGGAGCCCGTCAACGCGGCGGACGCAGCGGCCCTCGAGCACG

ACAAGGCCTACGACCAGCAGCTCCAAGCGGGTGACAATCCGTACCTGCGGTATAAT
CACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGTCTTTTGGGGGCAAC
CTCGGGCGCGCAGTCTTCCAGGCCAAAAAGCGGGTTCTCGAACCTCTGGGCCTGGTT
GAATCGCCGGTTAAGACGGCTCCTGGAAAGAAGAGACCGGTAGAGCCATCACCCCA
GCGCTCTCCAGACTCCTCTACGGGCATCGGCAAGAAAGGCCAGCAGCCCGCAAAAA
AGAGACTCAATTTTGGGCAGACTGGCGACTCAGAGTCAGTCCCCGACCCTCAACCAA
TCGGAGAACCACCAGCAGGCCCCTCTGGTCTGGGATCTGGTACAATGGCTGCAGGCG
GTGGCGCTCCAATGGCAGACAATAACGAAGGCGCCGACGGAGTGGGTAGTTCCTCA
GGAAATTGGCATTGCGATTCCACATGGCTGGGCGACAGAGTCATCACCACCAGCACC
CGCACCTGGGCCCTGCCCACCTACAACAACCACCTCTACAAGCAAATCTCCAACGGG
ACCTCGGGAGGAAGCACCAACGACAACACCTACTTCGGCTACAGCACCCCCTGGGG
GTATTTTGACTTCAACAGATTCCACTGCCACTTTTCACCACGTGACTGGCAGCGACTC
ATCAACAACAACTGGGGATTCCGGCCCAAGAGGCTCAACTTCAAGCTCTTCAACATC
CAAGTCAAGGAGGTCACGCAGAATGAAGGCACCAAGACCATCGCCAATAACCTTAC
CAGCACGATTCAGGTCTTTACGGACTCGGAATACCAGCTCCCGTACGTGCTCGGCTC
GGCGCACCAGGGCTGCCTGCCTCCGTTCCCGGCGGACGTCTTCATGATTCCTCAGTA
CGGGTACCTGACTCTGAACAATGGCAGTCAGGCTGTGGGCCGGTCGTCCTTCTACTG
CCTGGAGTACTTTCCTTCTCAAATGCTGAGAACGGGCAACAACTTTGAATTCAGCTA
CAACTTCGAGGACGTGCCCTTCCACAGCAGCTACGCGCACAGCCAGAGCCTGGACC
GGCTGATGAACCCTCTCATCGACCAGTACTTGTACTACCTGTCCCGGACTCAAAGCA
CGGGCGGTACTGCAGGAACTCAGCAGTTGCTATTTTCTCAGGCCGGGCCTAACAACA
TGTCGGCTCAGGCCAAGAACTGGCTACCCGGTCCCTGCTACCGGCAGCAACGCGTCT
CCACGACACTGTCGCAGAACAACAACAGCATCTTTGCCTGGACGGGTGCCACCAAGT
ATCATCTGAATGGCAGAGACTCTCTGGTGAATCCTGGCGTTGCCATGGCTACCCACA
AGGACGACGAAGAGCGATTTTTTCCATCCAGCGGAGTCTTAATGTTTGGGAAACAGG
GAGCTGGAAAAGACAACGTGGACTATAGCAGCGTGATGCTAACCAGCGAGGAAGAA
ATAAAGACCACCAACCCAGTGGCCACAGAACAGTACGGCGTGGTGGCCGATAACCT
GCAACAGCAAAACGCCGCTCCTATTGTAGGGGCCGTCAATAGTCAAGGAGCCTTACC
TGGCATGGTGTGGCAGAACCGGGACGTGTACCTGCAGGGTCCCATCTGGGCCAAGA
TTCCTCATACGGACGGCAACTTTCATCCCTCGCCGCTGATGGGAGGCTTTGGACTGA
AGCATCCGCCTCCTCAGATCCTGATTAAAAACACACCTGTTCCCGCGGATCCTCCGA
CCACCTTCAATCAGGCCAAGCTGGCTTCTTTCATCACGCAGTACAGTACCGGCCAGG
TCAGCGTGGAGATCGAGTGGGAGCTGCAGAAGGAGAACAGCAAACGCTGGAACCCA
GAGATTCAGTACACTTCCAACTACTACAAATCTACAAATGTGGACTTTGCTGTCAAT
ACTGAGGGTACTTATTCCGAGCCTCGCCCCATTGGCACCCGTTACCTCACCCGTAATC
TGTAA (**SEQ ID NO: 51**)

Amino Acid Sequence of AAVrh74W505R

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDNGRGLVLPGYKYLGPFN
GLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGNL
GRAVFQAKKRVLEPLGLVESPVKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPAKKRLN
FGQTGDSESVPDPQPIGEPPAGPSGLGSGTMAAGGGAPMADNNEGADGVGSSSGNWHC
DSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGSTNDNTYFGYSTPWGYFDFNRF
HCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFTDSE
YQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTG
NNFEFSYNFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQSTGGTAGTQQLLFSQAG
PNNMSAQAKNWLPGPCYRQQRVSTTLSQNNNSNFARTGATKYHLNGRDSLVNPGVAM
ATHKDDEERFFPSSGVLMFGKQGAGKDNVDYSSVMLTSEEEIKTTNPVATEQYGVVAD
NLQQQNAAPIVGAVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGL

KHPPPQILIKNTPVPADPPTTFNQAKLASFITQYSTGQVSVEIEWELQKENSKRWNPEIQY
TSNYYKSTNVDFAVNTEGTYSEPRPIGTRYLTRNL (**SEQ ID NO: 52**)

Nucleotide Sequence Encoding AAVrh74W505R

ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTCTCTGAGGGCATT
CGCGAGTGGTGGGACCTGAAACCTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAA
GCAGGACAACGGCCGGGGTCTGGTGCTTCCTGGCTACAAGTACCTCGGACCCTTCAA
CGGACTCGACAAGGGGGAGCCCGTCAACGCGGCGGACGCAGCGGCCCTCGAGCACG
ACAAGGCCTACGACCAGCAGCTCCAAGCGGGTGACAATCCGTACCTGCGGTATAAT
CACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGTCTTTTGGGGGCAAC
CTCGGGCGCGCAGTCTTCCAGGCCAAAAAGCGGGTTCTCGAACCTCTGGGCCTGGTT
GAATCGCCGGTTAAGACGGCTCCTGGAAAGAAGAGACCGGTAGAGCCATCACCCCA
GCGCTCTCCAGACTCCTCTACGGGCATCGGCAAGAAAGGCCAGCAGCCCGCAAAAA
AGAGACTCAATTTTGGGCAGACTGGCGACTCAGAGTCAGTCCCCGACCCTCAACCAA
TCGGAGAACCACCAGCAGGCCCCTCTGGTCTGGGATCTGGTACAATGGCTGCAGGCG
GTGGCGCTCCAATGGCAGACAATAACGAAGGCGCCGACGGAGTGGGTAGTTCCTCA
GGAAATTGGCATTGCGATTCCACATGGCTGGGCGACAGAGTCATCACCACCAGCACC
CGCACCTGGGCCCTGCCCACCTACAACAACCACCTCTACAAGCAAATCTCCAACGGG
ACCTCGGGAGGAAGCACCAACGACAACACCTACTTCGGCTACAGCACCCCCTGGGG
GTATTTTGACTTCAACAGATTCCACTGCCACTTTTCACCACGTGACTGGCAGCGACTC
ATCAACAACAACTGGGGATTCCGGCCCAAGAGGCTCAACTTCAAGCTCTTCAACATC
CAAGTCAAGGAGGTCACGCAGAATGAAGGCACCAAGACCATCGCCAATAACCTTAC
CAGCACGATTCAGGTCTTTACGGACTCGGAATACCAGCTCCCGTACGTGCTCGGCTC
GGCGCACCAGGGCTGCCTGCCTCCGTTCCCGGCGGACGTCTTCATGATTCCTCAGTA
CGGGTACCTGACTCTGAACAATGGCAGTCAGGCTGTGGGCCGGTCGTCCTTCTACTG
CCTGGAGTACTTTCCTTCTCAAATGCTGAGAACGGGCAACAACTTTGAATTCAGCTA
CAACTTCGAGGACGTGCCCTTCCACAGCAGCTACGCGCACAGCCAGAGCCTGGACC
GGCTGATGAACCCTCTCATCGACCAGTACTTGTACTACCTGTCCCGGACTCAAAGCA
CGGGCGGTACTGCAGGAACTCAGCAGTTGCTATTTTCTCAGGCCGGGCCTAACAACA
TGTCGGCTCAGGCCAAGAACTGGCTACCCGGTCCCTGCTACCGGCAGCAACGCGTCT
CCACGACACTGTCGCAGAACAACAACAGCAACTTTGCCAGGACGGGTGCCACCAAG
TATCATCTGAATGGCAGAGACTCTCTGGTGAATCCTGGCGTTGCCATGGCTACCCAC
AAGGACGACGAAGAGCGATTTTTTCCATCCAGCGGAGTCTTAATGTTTGGGAAACAG
GGAGCTGGAAAAGACAACGTGGACTATAGCAGCGTGATGCTAACCAGCGAGGAAGA
AATAAAGACCACCAACCCAGTGGCCACAGAACAGTACGGCGTGGTGGCCGATAACC
TGCAACAGCAAAACGCCGCTCCTATTGTAGGGGCCGTCAATAGTCAAGGAGCCTTAC
CTGGCATGGTGTGGCAGAACCGGGACGTGTACCTGCAGGGTCCCATCTGGGCCAAG
ATTCCTCATACGGACGGCAACTTTCATCCCTCGCCGCTGATGGGAGGCTTTGGACTG
AAGCATCCGCCTCCTCAGATCCTGATTAAAAACACACCTGTTCCCGCGGATCCTCCG
ACCACCTTCAATCAGGCCAAGCTGGCTTCTTTCATCACGCAGTACAGTACCGGCCAG
GTCAGCGTGGAGATCGAGTGGGAGCTGCAGAAGGAGAACAGCAAACGCTGGAACCC
AGAGATTCAGTACACTTCCAACTACTACAAATCTACAAATGTGGACTTTGCTGTCAA
TACTGAGGGTACTTATTCCGAGCCTCGCCCCATTGGCACCCGTTACCTCACCCGTAAT
CTGTAA (**SEQ ID NO: 53**)

CLAIMS

What is claimed is

1. An RNAi comprising a first strand and a second strand, wherein

a) the first strand and the second strand form a duplex;

b) the first strand comprises a guide region, wherein the guide region comprises nucleic acid with the sequence 5'- AGUCGAAGACAGUUCUAGGGU-3' (SEQ ID NO:1) or with a sequence with about 90% identity to the sequence of SEQ ID NO:1; and

c) the second strand comprises a non-guide region.

2. The RNAi of claim 1, wherein the non-guide region comprises nucleic acid with the sequence 5'-ACCCUAGAUGUCUUCGAUU-3' (SEQ ID NO:2) or a with a sequence with about 90% identity to the sequence of SEQ ID NO:2.

3. The RNAi of claim 1 or 2, wherein the first strand comprises nucleic acid with the sequence of SEQ ID NO:1 and the non-guide region comprises nucleic acid with the sequence of SEQ ID NO:2.

4. The RNAi of any one of claims 1-3, wherein the first strand and the second strand are linked by means of an RNA linker capable of forming a loop structure.

5. The RNAi of claim 4, wherein the RNA linker comprises from about 4 to about 50 nucleotides.

6. The RNAi of claim 4 or 5, wherein the loop structure comprises from about 4 to about 20 nucleotides.

7. The RNAi of any one of claims 4-6, wherein the loop structure comprises nucleic sequence with of SEQ ID NO:3 or with a sequence with about 90% identity to the sequence of SEQ ID NO:3.

8. The RNAi of any one of claims 4-7, wherein the RNAi comprises 5' to 3' the second strand, the RNA linker, and the first strand.

9. The RNAi of any one of claims 4-7, wherein the RNAi comprises 5' to 3' the first strand, the RNA linker, and the second strand.

10. The RNAi of any one of claims 1-8, wherein the RNAi comprises nucleic acid with the sequence of SEQ ID NO:7 or with a sequence with about 90% identity to the sequence of SEQ ID NO:7.

11. The RNAi of any one of claims 1-10, wherein the RNAi is a small inhibitory RNA (siRNA), a microRNA (miRNA), or a small hairpin RNA (shRNA).

12. The RNAi of any one of claim 1-11, wherein the RNAi further comprises a scaffold.

13. The RNAi of claim 12, wherein the scaffold comprises all or a portion of the nucleic acid of SEQ ID No: 11.

14. The RNAi of claim 13, wherein the miRNA is embedded within the scaffold.

15. The RNAi of claims 14, wherein the scaffold has a 5'arm, wherein the 5' arm is located 5' to the nucleic acid encoding the RNAi, and a 3'arm, wherein the 3' arm is located 3' to the nucleic acid encoding the RNAi.

16. The RNAi of any one of claims 12-15, wherein the scaffold is a miR-155 scaffold.

17. The RNAi of any one of claims 12-16, wherein the miR-155 scaffold comprises the nucleic acid of SEQ ID NO:9 or a sequence with about 90% identity to the sequence of SEQ ID NO:9 located 5' to the RNAi.

18. The RNAi of any one of claims 12-17, wherein the miR-155 scaffold comprises the nucleic acid of SEQ ID NO:10 or a sequence with about 90% identity to the sequence of SEQ ID NO:10 located 3' to the RNAi.

19. The RNAi of any one of claims 1-18, wherein the RNAi targets RNA encoding a polypeptide associated with myotonic dystrophy-1 (DM1).

20. The RNAi of claim 19, wherein the polypeptide is dystrophia myotonica protein kinase (DMPK).

21. The RNAi of claim 20, wherein the DMPK comprises a mutation associated with DM-1.

22. The RNAi of claim 20 or 21, wherein the gene encoding DMPK comprises five or more CTG trinucleotide repeats.

23. An expression cassette comprising nucleic acid encoding the RNAi of any one of claims 1-22.

24. The expression cassette of claim 23, wherein the nucleic acid encoding the RNAi is operably linked to a promoter.

25. The expression cassette of claim 24, wherein the promoter is a muscle-specific promotor.

26. The expression cassette of claim 24 or 25, wherein the promoter is a desmin promoter or variant thereof.

27. The expression cassette of claim 26, wherein the desmin promoter comprises one or more enhancer elements and the promoter for the human desmin gene.

28. The expression cassette of claim 26 or 27, wherein the desmin promoter comprises two enhancer elements and the promoter for the human desmin gene.

29. The expression cassette of any one of claims 26-28, wherein the desmin promoter comprises one or more Byrne enhancer elements and/or one or more Paulin enhancer elements.

30. The expression cassette of any one of claims 26-29, wherein the desmin promoter comprises one or more enhancer elements comprising the nucleotide sequence of SEQ ID NO:21 or a nucleotide sequence with about 90% identity to the sequence of SEQ ID NO:21

and/or one or more enhancer elements comprising the nucleotide sequence of SEQ ID NO:22 or a nucleotide sequence with about 90% identity to the sequence of SEQ ID NO:22.

31. The expression cassette of any one of claims 26-30, wherein the desmin promoter comprises the nucleotide sequence of SEQ ID NO:12 or a sequence with about 90% identity to the nucleotide sequence of SEQ ID NO:12.

32. The expression cassette of any one of claims 23-31, wherein the expression cassette further comprises an intron.

33. The expression cassette of claim 32, wherein the intron is a rabbit β-globin intron.

34. The expression cassette of claim 32 or 33, wherein the intron comprises the nucleotide sequence of SEQ ID NO:13 or a sequence with about 90% identity to the sequence of SEQ ID NO:13.

35. The expression cassette of any one of claims 32-34, wherein the nucleic acid encoding the RNAi is embedded in the intron.

36. The expression cassette of claim 35, wherein the intron comprises a 5' arm and a 3' arm, wherein the 5' arm is located 5' to the nucleic acid encoding the RNAi and the 3' arm is located 3' to the nucleic acid encoding the RNAi.

37. The expression cassette of claim 36, wherein the 5' arm of the intron comprises the nucleotide sequence of SEQ ID NO:14 or a sequence with about 90% identity to the sequence of SEQ ID NO:14.

38. The expression cassette of claim 36 or 37, wherein the 3' arm of the intron comprises the nucleotide sequence of SEQ ID NO:15 or a sequence with about 90% identity to the sequence of SEQ ID NO:15.

39. The expression cassette of any one of claims 23-38, wherein the expression cassette further comprises a polyadenylation signal.

40. The expression cassette of claim 39 wherein the polyadenylation signal is a bovine growth hormone polyadenylation signal, an SV40 polyadenylation signal, or a HSV TK pA.

41. The expression cassette of claim 40, wherein the polyadenylation signal is a minimal bovine growth hormone polyadenylation signal.

42. The expression cassette of any one of claims 39-41, wherein the bovine growth hormone polyadenylation signal comprises the nucleotide sequence of SEQ ID NO:16 or a sequence with about 90% identity to the sequence of SEQ ID NO:16.

43. The expression cassette of any one of claims 23-42, wherein the expression cassette comprises the nucleotide sequence of SEQ ID NO:17 or a sequence with about 90% identity to the sequence of SEQ ID NO:17.

44. An expression cassette, wherein the expression cassette comprises a modified desmin promoter, wherein the modified desmin promoter comprises one or more enhancer elements and the promoter for the human desmin gene.

45. The expression cassette of claim 44, wherein the modified desmin promoter comprises two enhancer elements and the promoter for the human desmin gene.

46. The expression cassette of claim 44 or 45, wherein the modified desmin promoter comprises one or more Byrne enhancer elements and/or one or more Paulin enhancer elements.

47. The expression cassette of any one of claims 44-46, wherein the modified desmin promoter comprises one or more enhancer elements comprising the nucleotide sequence of SEQ ID NO:21 or a nucleotide sequence with about 90% identity to the sequence of SEQ ID NO:21 and/or one or more enhancer elements comprising the nucleotide sequence of SEQ ID NO:22 or a nucleotide sequence with about 90% identity to the sequence of SEQ ID NO:22.

48. The expression cassette of any one of claims 44-47, wherein the desmin promoter comprises the nucleotide sequence of SEQ ID NO:12 or a sequence with about 90% identity to the nucleotide sequence of SEQ ID NO:12.

49. The expression cassette of any one of claims 44-48, wherein the expression cassette further comprises an intron.

50. The expression cassette of claim 49, wherein the intron is a rabbit β-globin intron.

51. The expression cassette of claim 49 or 50, wherein the intron comprises the nucleotide sequence of SEQ ID NO:13 or a sequence with about 90% identity to the sequence of SEQ ID NO:13.

52. The expression cassette of any one of claims 44-51, wherein the nucleic acid encoding the transgene is embedded in the intron.

53. The expression cassette of claim 52, wherein the intron comprises a 5' arm and a 3' arm, wherein the 5' arm is located 5' to the nucleic acid encoding the transgene and the 3' arm is located 3' to the nucleic acid encoding the transgene.

54. The expression cassette of claim 53, wherein the 5' arm of the intron comprises the nucleotide sequence of SEQ ID NO:14 or a sequence with about 90% identity to the sequence of SEQ ID NO:14.

55. The expression cassette of claim 53 or 54, wherein the 3' arm of the intron comprises the nucleotide sequence of SEQ ID NO:15 or a sequence with about 90% identity to the sequence of SEQ ID NO:15.

56. The expression cassette of any one of claims 44-55, wherein the expression cassette further comprises a polyadenylation signal.

57. The expression cassette of claim 56, wherein the polyadenylation signal is a bovine growth hormone polyadenylation signal, an SV40 polyadenylation signal, or a HSV TK pA.

58. The expression cassette of claim 57, wherein the polyadenylation signal is a minimal bovine growth hormone polyadenylation signal.

59. The expression cassette of any one of claims 56-58, wherein the bovine growth hormone polyadenylation signal comprises the nucleotide sequence of SEQ ID NO:16 or a sequence with about 90% identity to the sequence of SEQ ID NO:16.

60. The expression cassette of any one of claims 44-59, wherein the transgene encodes a polypeptide or a nucleic acid.

61. The expression cassette of any one of claims 44-60, wherein the transgene encodes an RNAi.

62. A vector comprising the expression cassette of any one of claims 23-61.

63. The vector of claim 62, wherein the expression cassette is flanked by one or more stuffer nucleic acid sequences.

64. The vector of claim 63, wherein the one or more stuffer nucleic acid sequences is derived from the human SerpinA1 gene.

65. The vector of claim 63 or 64, wherein a stuffer nucleic acid sequence located 5' to the expression cassette is derived from the human SerpinA1 gene.

66. The vector of any one of claims 63-65, wherein a stuffer sequence located 5' to the expression cassette comprises the nucleotide sequence of SEQ ID NO:18 or a sequence with about 90% identity to the sequence of SEQ ID NO:18.

67. The vector of any one of claims 63-66, wherein a stuffer nucleic acid sequence located 3' to the expression cassette is derived from the human SerpinA1 gene.

68. The vector of any one of claims 63-67, wherein a stuffer sequence located 3' to the expression cassette comprises the nucleotide sequence of SEQ ID NO:19 or a sequence with about 90% identity to the sequence of SEQ ID NO:19.

69. The vector of any one of claims 62-68, wherein the vector is a recombinant adeno-associated virus (rAAV) vector.

70. The rAAV vector of claim 69, wherein the expression cassette is flanked by one or more AAV inverted terminal repeat (ITR) sequences.

71. The rAAV vector of claim 70, wherein the expression cassette is flanked by two AAV ITRs.

72. The rAAV vector of claim 70 or 71, wherein the AAV ITRs are AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV2R471A, AAV DJ, a goat AAV, bovine AAV, or mouse AAV serotype ITRs.

73. The rAAV vector of any one of claims 70-72, wherein the AAV ITRs are AAV2 ITRs.

74. The rAAV vector of any one of claims 69-73, wherein the rAAV vector comprises the nucleotide sequence of SEQ ID NO:20 or a sequence with about 90% identity to the sequence of SEQ ID NO:20.

75. The rAAV vector of any one of claims 69-74, wherein the vector is a self-complementary rAAV vector.

76. A cell comprising the expression cassette of any one of claims 23-61, the vector of any one of claims 62-68, or the rAAV vector of any one of claims 69-75.

77. A viral particle comprising the vector of any one of claims 62-68.

78. A recombinant AAV particle comprising the rAAV vector of any one of claims 69-75.

79. The rAAV particle of claim 78, wherein the AAV viral particle comprises an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAVrh74, AAVrh74 N502I, AAVrh74 W505R, AAV2R471A, AAV2/2-7m8, AAV DJ, AAV2 N587A, AAV2 E548A, AAV2 N708A, AAV V708K, AAV2-HBKO, AAVDJ8, AAVPHP.B, AAVPHP.eB, AAVBR1, AAVHSC15, AAVHSC17,

a goat AAV, AAV1/AAV2 chimeric, bovine AAV, or mouse AAV capsid rAAV2/HBoV1 serotype capsid.

80. The rAAV particle of claim 78 or 79, wherein the ITR and the capsid of the rAAV viral particle are derived from the same AAV serotype.

81. The rAAV particle of claim 78 or 79, wherein the ITR and the capsid of the rAAV viral particle are derived from different AAV serotypes.

82. The rAAV particle of claim 78, 79 or 81, wherein the AAV viral particle comprises a AAVrh74 N502I serotype capsid.

83. The rAAV particle of claim 82, wherein the ITR is an AAV2 ITR and the capsid of the rAAV particle is an AAVrh74 N502I serotype capsid.

84. The rAAV particle of claim 78, 79, or 81, wherein the AAV viral particle comprises a AAVrh74 W505R serotype capsid.

85. The rAAV particle of claim 83a, wherein the ITR is an AAV2 ITR and the capsid of the rAAV particle is an AAVrh74 W505R serotype capsid.

86. An rAAV particle comprising an rAAV vector and a capsid, wherein the rAAV vector comprises the following nucleic acids 5' to 3',

an AAV2 ITR,
nucleic acid encoding a stuffer nucleic acid sequence from the human serpinA1 gene,
a Byrne desmin enhancer element,
a Paulin desmin enhancer element,
a desmin promoter,
a 5' arm of a rabbit β-globin intron,
a 5' miR155 scaffold sequence,
a $DMPK^{204}$ miRNA guide sequence,
a miR155 terminal loop sequence,
a $DMPK^{204}$ miRNA passenger sequence,
a 3' miR155 scaffold sequence,

a 3' arm of a rabbit β-globin intron,

a minimal bovine growth hormone polyadenylation sequence,

nucleic acid encoding a stuffer nucleic acid sequence from the human serpinA1 gene, and

an AAV2 ITR;

and wherein the capsid is an AAVrh74 N502I capsid.

87. An rAAV particle comprising an rAAV vector, wherein the rAAV vector comprises the following nucleic acids 5' to 3',

an AAV2 ITR comprising the polynucleotide sequence of SEQ ID NO:43,

nucleic acid encoding a stuffer nucleic acid sequence from the human serpinA1 gene comprising the polynucleotide sequence of SEQ ID NO:18,

a Byrne desmin enhancer element comprising the polynucleotide sequence of SEQ ID NO:21,

a Paulin desmin enhancer element comprising the polynucleotide sequence of SEQ ID NO:22,

a desmin promoter comprising the polynucleotide sequence of SEQ ID NO:23,

a 5' arm of a rabbit β-globin intron comprising the polynucleotide sequence of SEQ ID NO:14,

a 5' miR155 scaffold sequence comprising the polynucleotide sequence of SEQ ID NO:40,

a $DMPK^{204}$ miRNA guide sequence comprising the polynucleotide sequence of SEQ ID NO:4,

a miR155 terminal loop sequence comprising the polynucleotide sequence of SEQ ID NO:6,

a $DMPK^{204}$ miRNA passenger sequence comprising the polynucleotide sequence of SEQ ID NO:5,

a 3' miR155 scaffold sequence comprising the polynucleotide sequence of SEQ ID NO:41,

a 3' arm of a rabbit β-globin intron comprising the polynucleotide sequence of SEQ ID NO:15,

a minimal bovine growth hormone polyadenylation sequence comprising the polynucleotide sequence of SEQ ID NO:16,

nucleic acid encoding a stuffer nucleic acid sequence from the human serpinA1 gene comprising the polynucleotide sequence of SEQ ID NO:19, and

an AAV2 ITR comprising the polynucleotide sequence of SEQ ID NO:49;

and wherein the capsid is an AAVrh74 N502I capsid.

88. The rAAV particle of claim 86 or 87, wherein the AAVrh74 N502I capsid comprises capsid proteins comprising the amino acid sequence of SEQ ID NO:50.

89. An rAAV particle comprising an rAAV vector and a capsid, wherein the rAAV vector comprises the following nucleic acids 5' to 3', an AAV2 ITR, nucleic acid encoding a stuffer nucleic acid sequence from the human serpinA1 gene, a Byrne desmin enhancer element, a Paulin desmin enhancer element, a desmin promoter, a 5' arm of a rabbit β-globin intron, a 5' miR155 scaffold sequence, a $DMPK^{204}$ miRNA guide sequence, a miR155 terminal loop sequence, a $DMPK^{204}$ miRNA passenger sequence, a 3' miR155 scaffold sequence, a 3' arm of a rabbit β-globin intron, a minimal bovine growth hormone polyadenylation sequence, nucleic acid encoding a stuffer nucleic acid sequence from the human serpinA1 gene, and an AAV2 ITR; and wherein the capsid is an AAVrh74 W505R capsid.

90. An rAAV particle comprising an rAAV vector, wherein the rAAV vector comprises the following nucleic acids 5' to 3', an AAV2 ITR comprising the polynucleotide sequence of SEQ ID NO:43, nucleic acid encoding a stuffer nucleic acid sequence from the human serpinA1 gene comprising the polynucleotide sequence of SEQ ID NO:18, a Byrne desmin enhancer element comprising the polynucleotide sequence of SEQ ID NO:21, a Paulin desmin enhancer element comprising the polynucleotide sequence of SEQ ID NO:22, a desmin promoter comprising the polynucleotide sequence of SEQ ID NO:23, a 5' arm of a rabbit β-globin intron comprising the polynucleotide sequence of SEQ ID NO:14, a 5' miR155 scaffold sequence comprising the polynucleotide sequence of SEQ ID NO:40, a $DMPK^{204}$ miRNA guide sequence comprising the polynucleotide sequence of SEQ ID NO:4, a miR155 terminal loop sequence comprising the polynucleotide sequence of SEQ ID NO:6, a $DMPK^{204}$ miRNA passenger sequence comprising the polynucleotide sequence of SEQ ID NO:5, a 3' miR155 scaffold sequence comprising the polynucleotide sequence of SEQ ID NO:41, a 3' arm of a rabbit β-globin intron comprising the polynucleotide sequence of SEQ ID NO:15, a minimal bovine growth hormone polyadenylation sequence comprising the polynucleotide sequence of SEQ ID NO:16, nucleic acid encoding a stuffer nucleic acid

sequence from the human serpinA1 gene comprising the polynucleotide sequence of SEQ ID NO:19, and an AAV2 ITR comprising the polynucleotide sequence of SEQ ID NO:49; and wherein the capsid is an AAVrh74 W505R capsid.

91. The rAAV particle of claim 86a or 86b, wherein the AAVrh74 W505R capsid comprises capsid proteins comprising the amino acid sequence of SEQ ID NO: 52.

92. A composition comprising the viral particle claim 77 or the rAAV particle of any one of claims 78-86.

93. A pharmaceutical composition comprising the viral particle claim 77 or the rAAV particle of any one of claims 78-86.

94. The composition of claim 87 or 88, wherein the composition further comprises a pharmaceutically acceptable carrier.

95. A modified desmin promoter, wherein the modified desmin promoter comprises one or more enhancer elements and the promoter for the human desmin gene.

96. The modified desmin promoter of claim 90, wherein the modified desmin promoter comprises two enhancer elements and the promoter for the human desmin gene.

97. The modified desmin promoter of claim 90 or 91, wherein the modified desmin promoter comprises one or more Byrne enhancer elements and/or one or more Paulin enhancer elements.

98. The modified desmin promoter of any one of claims 90-92, wherein the modified desmin promoter comprises one or more enhancer element comprising the nucleotide sequence of SEQ ID NO:21 or a sequence with about 90% identity to the sequence of SEQ ID NO:21 and/or one or more enhancer element comprising the nucleotide sequence of SEQ ID NO:22 or a sequence with about 90% identity to the sequence of SEQ ID NO:22.

99. The modified desmin promoter of any one of claims 90-93, wherein the modified desmin promoter comprises the nucleotide sequence of SEQ ID NO:12 or a sequence with about 90% identity to the sequence of SEQ ID NO:12.

100. A kit comprising the RNAi of any one of claims 1-22.

101. A kit comprising the viral particle of claim 77 or the AAV particle of any one of claims 78-86.

102. A kit comprising the composition of any one of claims 87-89.

103. The kit of any one of claims 95-97, further comprising instructions for use.

104. A method for treating myotonic dystrophy-1 (DM1) in a mammal in need thereof comprising administering to the mammal an effective amount of the RNAi of any one of claims 1-22.

105. A method for inhibiting the expression of dystrophia myotonica protein kinase (DMPK) in a mammal with DM-1 in need thereof comprising administering to the mammal an effective amount of the RNAi of any one of claims 1-22.

106. A method for inhibiting the accumulation of DMPK RNA in a cell of a mammal with DM-1 in need thereof comprising administering to the mammal an effective amount of the RNAi of any one of claims 1-22.

107. A method for treating myotonic dystrophy-1 (DM1) in a mammal in need thereof comprising administering to the mammal an effective amount of the viral particle of claim 77 or the rAAV particle of any one of claims 78-86.

108. A method for inhibiting the expression of dystrophia myotonica protein kinase (DMPK) in a mammal with DM-1 in need thereof comprising administering to the mammal an effective amount of the viral particle of claim 77 or an effective amount of the rAAV particle of any one of claims 78-86.

109. A method for inhibiting the accumulation of DMPK RNA in a cell of a mammal with DM-1 in need thereof comprising administering to the mammal an effective amount of the

viral particle of claim 77 or an effective amount of the rAAV particle of any one of claims 78-86.

110. The method of any one of claims 102-104, wherein the effective amount of the viral particle or rAAV particle is a dose of about 1 x 10^{8} to about 2 x 10^{13} genome copies/mL.

111. The method of claim 105, wherein the dose is about 5 x 10^{12} genome copies/mL.

112. The method of claim 105, wherein the dose is about 1 x 10^{13} genome copies/mL.

113. The method of claim 105, wherein the dose is about 2 x 10^{13} genome copies/mL.

114. The method of any one of claims 102-104, wherein the effective amount of the viral particle or rAAV particle is a dose of about 1 x 10^{8} to about 2 x 10^{14} genome copies/kg of body weight.

115. The method of claim 109, wherein the dose is about 5 x 10^{13} genome copies/kg of body weight.

116. The method of claim 109, wherein the dose is about 1 x 10^{14} genome copies/kg of body weight.

117. The method of claim 109, wherein the dose is about 2 x 10^{14} genome copies/kg of body weight.

118. A method for treating myotonic dystrophy-1 (DM1) in a mammal in need thereof comprising administering to the mammal an effective amount of the composition of any one of claims 87-89.

119. A method for inhibiting the expression of dystrophia myotonica protein kinase (DMPK) in a mammal with DM-1 in need thereof comprising administering to the mammal an effective amount of the composition of any one of claims 87-89.

120. A method for inhibiting the accumulation of DMPK RNA in a cell of a mammal with DM-1 in need thereof comprising administering to the mammal an effective amount of the composition of any one of claims 87-89.

121. The method of any one of claims 99-101, wherein the RNAi is administered in combination with an immunosuppressive agent, wherein the immunosuppressive agent is administered before, at the same time, and/or after administration of the RNAi.

122. The method of any one of claims 102-104, wherein the viral particle or the rAAV particle is administered in combination with an immunosuppressive agent, wherein the immunosuppressive agent is administered before, at the same time, and/or after administration of the viral particle or the rAAV particle.

123. The method of any one of claims 113-115, wherein the composition is administered in combination with an immunosuppressive agent, wherein the immunosuppressive agent is administered before, at the same time, and/or after administration of the composition.