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); 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).

3. The RNAi of claim 1 or 2, wherein the first strand and the second strand are linked by means of an RNA linker capable of forming a loop structure;

optionally wherein the RNA linker comprises from about 4 to about 50 nucleotides and/or wherein the loop structure comprises from about 4 to about 20 nucleotides;

optionally wherein the loop structure comprises the nucleic sequence of SEQ ID NO:3 or comprises a sequence with about 90% identity to the sequence of SEQ ID NO:3;

optionally wherein the RNAi comprises 5' to 3' the second strand, the RNA linker, and the first strand, or wherein the RNAi comprises 5' to 3' the first strand, the RNA linker, and the second strand;

optionally wherein the RNAi comprises a 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; and/or

optionally wherein the RNAi is a small inhibitory RNA (siRNA), a microRNA (miRNA), or a small hairpin RNA (shRNA).

4. The RNAi of any one of claim 1-3, wherein the RNAi further comprises a scaffold,

optionally wherein the scaffold comprises all or a portion of the nucleic acid of SEQ ID No: 11;

optionally wherein the miRNA is embedded within the scaffold;

optionally 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;

optionally wherein the scaffold is a miR-155 scaffold; and/or

optionally 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, or 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.

5. The RNAi of any one of claims 1-4, wherein the RNAi targets RNA encoding a polypeptide associated with myotonic dystrophy-1 (DM1);

optionally wherein the polypeptide is dystrophia myotonica protein kinase (DMPK);

optionally wherein the DMPK comprises a mutation associated with DM-1; and/or

optionally wherein the gene encoding DMPK comprises five or more CTG trinucleotide repeats.

6. An expression cassette comprising nucleic acid encoding the RNAi of any one of claims 1-5;

optionally wherein the nucleic acid encoding the RNAi is operably linked to a promoter;

optionally wherein the promoter is a muscle-specific promotor;

optionally wherein the promoter is a desmin promoter or variant thereof;

optionally wherein the desmin promoter comprises one or more enhancer elements and the promoter for the human desmin gene;

optionally wherein the desmin promoter comprises two enhancer elements and the promoter for the human desmin gene; and/or

optionally wherein the desmin promoter comprises one or more Byrne enhancer elements and/or one or more Paulin enhancer elements.

7. The expression cassette of claim 6, 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;

optionally 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;

optionally wherein the expression cassette further comprises an intron;

optionally wherein the intron is a rabbit β-globin intron;

optionally 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;

optionally wherein the nucleic acid encoding the RNAi is embedded in the intron;

optionally 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;

optionally 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;

optionally 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;

optionally wherein the expression cassette further comprises a polyadenylation signal;

optionally wherein the polyadenylation signal is a bovine growth hormone polyadenylation signal, an SV40 polyadenylation signal, or a HSV TK pA;

optionally wherein the polyadenylation signal is a minimal bovine growth hormone polyadenylation signal;

optionally 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; and/or

optionally 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.

8. 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;

optionally wherein the modified desmin promoter comprises two enhancer elements and the promoter for the human desmin gene;

optionally wherein the modified desmin promoter comprises one or more Byrne enhancer elements and/or one or more Paulin enhancer elements;

optionally 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.

9. The expression cassette of claim 8, 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;

optionally wherein the expression cassette further comprises an intron;

optionally wherein the intron is a rabbit β-globin intron;

optionally 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;

optionally wherein the nucleic acid encoding the transgene is embedded in the intron;

optionally 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;

optionally 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;

optionally 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;

optionally wherein the expression cassette further comprises a polyadenylation signal;

optionally wherein the polyadenylation signal is a bovine growth hormone polyadenylation signal, an SV40 polyadenylation signal, or a HSV TK pA;

optionally wherein the polyadenylation signal is a minimal bovine growth hormone polyadenylation signal;

optionally 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;

optionally wherein the transgene encodes a polypeptide or a nucleic acid; and/or

optionally wherein the transgene encodes an RNAi.

10. A vector comprising the expression cassette of any one of claims 6-9, wherein the vector is a recombinant adeno-associated virus (rAAV) vector;

optionally wherein the expression cassette is flanked by one or more stuffer nucleic acid sequences;

optionally wherein the one or more stuffer nucleic acid sequences are derived from the human SerpinA1 gene;

optionally wherein a stuffer nucleic acid sequence located 5' to the expression cassette is derived from the human SerpinA1 gene;

optionally 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;

optionally wherein a stuffer nucleic acid sequence located 3' to the expression cassette is derived from the human SerpinA1 gene;

optionally 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;

optionally wherein the expression cassette is flanked by one or more AAV inverted terminal repeat (ITR) sequences;

optionally wherein the expression cassette is flanked by two AAV ITRs;

optionally 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;

optionally wherein the AAV ITRs are AAV2 ITRs;

optionally 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; and/or

optionally wherein the vector is a self-complementary rAAV vector.

11. A cell comprising the expression cassette of claim 6 or 7, or the vector of claim 10, or a viral particle comprising the vector of claim 10.

12. A recombinant AAV (rAAV) particle comprising the rAAV vector of claim 10.

13. The rAAV particle of claim 12, wherein the rAAV viral particle comprises an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAVrh74, 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;

optionally wherein the ITR and the capsid of the rAAV viral particle are derived from the same AAV serotype;

optionally wherein the ITR and the capsid of the rAAV viral particle are derived from different AAV serotypes;

optionally wherein the AAV viral particle comprises an AAVrh74 W505R serotype capsid; and/or

optionally wherein the ITR is an AAV2 ITR and the capsid of the rAAV particle is an AAVrh74 W505R serotype capsid.

14. 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,

a 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,

a 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.

15. 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,

a 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,

a 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.

16. 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,

a 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,
a 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.

17. The rAAV particle of claim 15 or 16, wherein the AAVrh74 W505R capsid comprises capsid proteins comprising the amino acid sequence of SEQ ID NO: 52.

18. A composition or pharmaceutical composition comprising the rAAV particle of any one of claims 12-17,

optionally wherein the composition further comprises a pharmaceutically acceptable carrier.

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

optionally wherein the modified desmin promoter comprises two enhancer elements and the promoter for the human desmin gene;

optionally wherein the modified desmin promoter comprises one or more Byrne enhancer elements and/or one or more Paulin enhancer elements;

optionally 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;

optionally 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.

20. A kit comprising the RNAi of any one of claims 1-5, or the rAAV particle of any one of claims 12-17, or the composition of claim 18;

optionally further comprising instructions for use.

21. Use of the RNAi of any one of claims 1-5 for:

a) the manufacture of a medicament for treating myotonic dystrophy-1 (DM1) in a mammal in need thereof;

b) the manufacture of a medicament for inhibiting the expression of dystrophia myotonica protein kinase (DMPK) in a mammal with DM-1 in need thereof; and/or

c) the manufacture of a medicament for inhibiting the accumulation of DMPK RNA in a cell of a mammal with DM-1 in need thereof;

optionally wherein the RNAi is to be administered in combination with an immunosuppressive agent, wherein the immunosuppressive agent is to be administered before, at the same time, and/or after administration of the RNAi.

22. Use of the rAAV particle of any one of claims 12-17 for:

a) the manufacture of a medicament for treating myotonic dystrophy-1 (DM1) in a mammal in need thereof;

b) the manufacture of a medicament for inhibiting the expression of dystrophia myotonica protein kinase (DMPK) in a mammal with DM-1 in need thereof; and/or

c) the manufacture of a medicament for inhibiting the accumulation of DMPK RNA in a cell of a mammal with DM-1 in need thereof;

optionally wherein the rAAV particle is to be administered in combination with an immunosuppressive agent, wherein the immunosuppressive agent is to be administered before, at the same time, and/or after administration of the rAAV particle.

23. The use of claim 21 or 22, 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,

optionally wherein the dose is about 5 x $10^{12}$ genome copies/mL,

optionally wherein the dose is about 1 x $10^{13}$ genome copies/mL, or

optionally wherein the dose is about 2 x $10^{13}$ genome copies/mL.

24. The use of claim 21 or 22, 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,

optionally wherein the dose is about 5 x $10^{13}$ genome copies/kg of body weight,

optionally wherein the dose is about 1 x $10^{14}$ genome copies/kg of body weight,

optionally wherein the dose is about 2 x $10^{14}$ genome copies/kg of body weight.

25. Use of the composition of claim 18 for:

a) the manufacture of a medicament for treating myotonic dystrophy-1 (DM1) in a mammal in need thereof,

b) the manufacture of a medicament for inhibiting the expression of dystrophia myotonica protein kinase (DMPK) in a mammal with DM-1 in need thereof, and/or

c) the manufacture of a medicament for inhibiting the accumulation of DMPK RNA in a cell of a mammal with DM-1 in need thereof;

optionally wherein the composition is to be administered in combination with an immunosuppressive agent, wherein the immunosuppressive agent is to be administered before, at the same time, and/or after administration of the composition.